The Sea Cucumber *Apostichopus japonicus*
History, Biology and Aquaculture

Edited by

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Preface

Sea cucumbers are one of the most important marine resources supporting coastal livelihoods around the world. They are widely distributed, from the shores to deep ocean trenches, inhabit diverse habitats, and often exhibit large biomasses. The Western Pacific coast hosts a rich diversity of sea cucumbers, among which *Apostichopus japonicus* is a focal species.

Sea cucumbers, and *A. japonicus* in particular, are closely woven into the history and culture of China and neighboring East Asian countries. The Chinese character "参," which refers to sea cucumber, can be traced back to the Oracle Bone inscriptions from the Shang Dynasty, 1300 B.C. The first record of sea cucumber consumption is found as early as the Three Kingdoms Period. For hundreds of years, sea cucumber has been considered among the most nutritious foods in China; it is now a prized delicacy alongside the well-known birds' nests and abalones. As such, it is at the forefront of many regional cuisines of China, especially at important banquets. The medicinal properties of sea cucumber are recorded in famous Chinese books such as "A Supplement to the Compendium of Materia Medica" and "New Compilation of Materia Medica." The singularities and significance of sea cucumber are also recorded in Chinese literature. Many folk tales highlight its special morphological characters and tonic effects.

Today, *A. japonicus* has incontestable economic value and potential; it is at the heart of a huge industry worth over 5 billion USD. This species exhibits many fascinating biological characters such as aestivation, evisceration, regeneration, albinism, and autolysis. The peculiar biological features, mythical curative properties, and unique traditional culture surrounding *A. japonicus* have inspired our research interests.

Why has the sea cucumber played such a long-standing and important role in Chinese traditional culture? Why do the Chinese love eating sea cucumber so much? What curative effects does it really have? How is sea cucumber best cooked? What is the best aquaculture model for sea cucumber? How can environmentally friendly and sustainable development of the sea cucumber industry be achieved?

For many years, research groups at the Institute of Oceanology, Chinese Academy of Sciences (IOCAS) have carried out studies on the biology, physiology, ethology, and aquaculture of *A. japonicus*. With our growing knowledge of *A. japonicus*, we became even fonder of this ancient and mysterious animal. The idea to compile this knowledge in a book first emerged in November 2011, following an academic exchange with Canadian colleagues who were equally fascinated with sea cucumbers. Our discussions were both informative and fruitful. They expressed a desire to help us disseminate findings that were largely confined to Chinese publications, for the benefit of researchers and industries who were preoccupied with *A. japonicus* and other species of sea cucumber in the rest of the world. The draft outline of the book was prepared in March 2012, and the chapters fleshed out during our visit to Canada in September 2012. Invitations were thereafter extended to colleagues outside IOCAS with compatible interests, to capture the full scope of research areas surrounding *A. japonicus*. Given the language barrier, it was admittedly challenging to summarize the biological studies, the development of the aquaculture industry and to present both the traditional and modern culture methods of *A. japonicus*. The Chinese contributors prepared the initial drafts either in their native language or in tentative English. Each chapter was then revised internally at IOCAS until a full preliminary English version was obtained

in May 2013. It took another 15 months for my English-speaking co-editors to lead contributors through several additional rounds of revision, until the book was finally ready.

Special thanks should be given to my esteemed co-editors, who put a lot of time and effort into this book. It was quite an undertaking! They not only planned the structure of the book with us, but also invited researchers from Democratic People's Republic of Korea, Republic of Korea, and Japan to take part in the project. I was impressed by their rigorous and careful editing, great attitude, and hard work. Without their efforts, the book could not have been completed and published successfully.

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Of all the creatures humans consume, sea cucumbers might well qualify as the weirdest. Worm-shaped and slimy, smooth or horny, with no clear front and rear ends and no easily discernible movements, these ocean dwellers are anything but endearing. The fact that many of them feed on detritus does nothing to improve their image. Yet, in their infinite wisdom, East Asian people have marked sea cucumbers as one of our planet's most valuable gifts, with such emphasis that sea cucumber is one of the most prized seafood in the world today.

Among the approximately 1200 known species of sea cucumber, some 70 are harvested worldwide. But the undisputed favorite, the so-called king of sea cucumbers, is *Apostichopus japonicus*, sometimes referred to in English literature as the Japanese sea cucumber. This species is not larger or more colorful than others, but its native populations extend along the coasts of the Northwest Pacific, where the love of sea cucumber first bloomed. A millennium later, this adoration has taken its toll; with a market value peaking close to 3000 USD per dry kilo, *A. japonicus* is now officially an endangered species, as most of its wild populations are dwindling dangerously. On the other hand, there is no shortage of captive-bred specimens in China, where sea cucumber is now the number one aquaculture industry.

The Chinese people's enduring relationship with sea cucumber has had far-reaching repercussions over the past century. On a planetary scale, it has led to overfishing of most commercial species, some to the brink of extinction, followed by countless initiatives to breed sea cucumbers of every denomination across the globe. All this in an effort to sustain the mythical appetite of 1.5 billion people living in China, in neighboring East Asian nations and in Chinatowns worldwide. Nearly everywhere we go, we meet people involved in sea cucumber conservation or production initiatives, from remote islands of the Indo-Pacific, to coastal nations of South and Central America, Asia, and Africa, all the way to Russia and North America, including Newfoundland, where we currently live.

Our own fascination with sea cucumbers was born out of curiosity for their strange looks and behaviors. We initially strove to understand how they reproduce, feed, and communicate with each other, and to assess the role they play in marine ecosystems from shallow to deep-sea environments. Our fundamental interest in their biology eventually drove us toward more applied projects, first in Canada and then abroad, as our expertise was sought to assist fisheries and aquaculture ventures. To this day, all things sea cucumber still guide our travels around the world.

We suspect that *A. japonicus* is the most studied sea cucumber by a fair margin, and perhaps even the most studied echinoderm. The number of English publications on this species is already high, without counting those in Chinese, Japanese, Korean, and Russian. This led us to ponder how much information lay underused and underappreciated in foreign-language or gray literature. Hence came the idea to ask our Chinese colleagues to share their knowledge with us through this book. Our initial aim was to fulfill the curiosity of sea cucumber aficionados and researchers like us and to help stakeholders around the world understand the cultural background and success of China when it comes to sea cucumber production and trade.

We have tried to solicit contributions from experts in nearly all aspects of the biology, ecology, captive breeding, and commerce of *A. japonicus*. Admittedly and despite our best efforts, this book is not comprehensive; we recognize that much more information could have been included. Notwithstanding space constraints, emphasis was definitely placed on information that had not previously been published in English. We broke a number of language barriers, but certainly not all of them. The initial focus was on China; ultimately, Japan and the two Koreas joined in. In all, 42 contributors from 16 institutions and six countries were directly involved in this project (aside from external collaborators and reviewers; see Acknowledgments). We take this opportunity to apologize for the imprecisions and inaccuracies

that have doubtless evaded our scrutiny. Please be reminded that they are not necessarily the fault of the contributors, but perhaps an unfortunate outcome of their meaning being lost in translation.

The world of sea cucumber research is an ever-evolving one and we hope that this contribution will inspire and fuel many investigations to come!

The adventure of this book was a challenging yet rewarding one. We wish to warmly thank all the contributors for sharing their insight, for working hard over the course of nearly two years, and for replying quickly to our many requests, comments, and suggestions. Of course, none of this would have been possible without the support and dedication of our friend and co-editor Hongsheng Yang, and of his team. May the sea cucumber god grant them wisdom and prosperity in this life and beyond!

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CHAPTER

Apostichopus japonicus IN THE LIFE OF CHINESE PEOPLE

1

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SUMMARY

Holothurians or sea cucumbers, especially *Apostichopus japonicus*, have been part of the diet of the Chinese people for centuries; they are also woven into the fabric of Chinese culture as one of the most important foods. From legends to real facts, and from ancient emperor dynasties to the present day, this chapter covers the drivers of the sea cucumber industry in China and tries to explain why such tremendous demand exists worldwide for an unassuming and strange-looking marine invertebrate. The origin, evolution, and distribution of *A. japonicus* are also introduced, together with its proven and putative medicinal properties.

Keywords: ancient tale; *Apostichopus japonicus*; calligraphy; China; Chinese diet; medicinal value; nutritional value; poem; sea cucumber

1.1 ANCIENT TALES OF SEA CUCUMBER

Due to its powerful symbolism and medicinal properties, the sea cucumber is the subject of many Chinese legends and tales that have been passed down from generation to generation.

1.1.1 XU FU HUNTED FOR SEA CUCUMBER

In 219 BC, the First Emperor of China traveled around the Shandong Peninsula by boat, and stayed there for three months. He heard that there were three Fairy Mountains in Bohai Bay; they were Penglai, Fangzhang, and Yingzhou, where three immortals holding everlasting elixirs lived.

The man who told the mystical story to the First Emperor was named Xu Fu; he was a magician. It is said that Xu Fu had seen the three Fairy Mountains himself. The First Emperor was very glad after hearing what Xu Fu said, and he assigned him to search for the everlasting elixirs in the sea (Figure 1.1). Xu Fu led 3000 virgin boys and girls and "craftsmen," as well as bringing the "seeds of five cereals," on a hunt for the everlasting elixirs by boat. However, after being adrift at sea for a very long time, he did not find the Fairy Mountains, the immortals, or the everlasting elixirs.

Xu Fu failed to complete the task and was afraid to return because the First Emperor, well known to be cruel and ruthless, might decide to kill him. What was worse, Xu Fu had run out of food during his travels, and he had to make a living by fishing. To do so, he led the virgin girls and boys to

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FIGURE 1.1

The statues in Langyatai Scenic Spot, Qingdao, Shandong, China, depict the First Emperor of China sending Xu Fu in search of the everlasting elixirs in the sea.

Photo by Hongsheng Yang and Yucen Bai

a small island where they could live. While Xu Fu lived on the island, he found a very ugly marine organism, which is called sea cucumber in the modern language. Xu Fu decided to taste the "freak creature," so he asked his subordinates to cook it. The cooked sea cucumber smelled very good, which whetted Xu Fu's appetite greatly. Its taste was very smooth and delicious, which won repeated praise from Xu Fu. Following this, Xu Fu ordered his subordinates to collect sea cucumbers every day. Several days after eating sea cucumbers, Xu Fu felt that the *qi* ran freely in his body, and he became very vigorous. From then on, he decided to eat sea cucumbers every day, and he lived on the island like this for over 50 years. When he was almost 90 years old, he still looked very young, his beard and hair were black, and he was free from any diseases. Xu Fu finally understood that the sea cucumber was the "everlasting elixir." He called the sea cucumber 土肉 (Mud Meat) because its color was just like the color of mud.

Xu Fu assigned someone to send sea cucumbers to the First Emperor, not knowing that he had been dead for a long time. When Xu Fu learned that the emperor had already died, he sighed and said "If I had known the mud meat (sea cucumber) has such a magical efficacy earlier, he would not have died!"

1.1.2 HAI SHENG SAVED HIS PARENTS

Over a thousand years ago, a couple lived by the sea, and they lived off fishing. The woman gave birth to a boy who the couple called Hai Sheng. The family had to work hard to survive, and lived like this year after year. When Hai Sheng reached 15 years of age, his father developed a strange disease that made him feeble and unable to work. Therefore, the young boy had to take over the fishing. Every day, after fishing, the boy would go to the mountains to gather medicinal herbs in order to treat his father, and would then help his mother with housework. After half a year, the father did not get better. Even worse, the mother developed the same disease. As a result, Hai Sheng had to work day and night.

One day, Hai Sheng wearily rowed his boat out to fish again. After casting his net, he lay in the boat and fell asleep. In his sleep, he saw an old man with a white beard. The old man told Hai Sheng that only a species in the sea could cure his parents. Hai Sheng asked for the name of the creature and what it looked like. The old man smiled and said the species was very ugly, black like a worm, and with four rows of thorns on its body. Finally, the old man told Hai Sheng that the species could be found in Taozi Bay in the Bohai Sea. Before Hai Sheng could say more, he suddenly felt cold, woke up, and realized that he had had a dream.

Just then, the wind started to blow. Hai Sheng drew in the net and got much more fish than usual. After returning home, Hai Sheng told his parents about his dream. After consultation, the family decided to move. They sold everything they had, keeping only the fishing boat and nets, and then they left. Hai Sheng rowed the boat carrying his parents along the shore, asking any passersby if Taozi Bay was along the way. After a year, they finally discovered its location. Once there, Hai Sheng looked around as he walked, and saw a thatched cottage on the beach. As Hai Sheng reached the cottage, an old man emerged from it. Hai Sheng went to the old man and asked him about the creature. The old man replied, “The creature does exist, but nobody here dares to eat it.” The next day, Hai Sheng went to sea and captured two specimens. He cooked them for his parents to eat. Every day, Hai Sheng fished more for his parents. After three months, a miracle occurred; his parents got better and could work again. The father asked Hai Sheng how they should call the creature. Hai Sheng replied, “It does not have a name, but I call it seagod (海神; Haishen) because it lives in the sea, and can relieve people from diseases.”

Soon, the emperor heard the story, and dispatched people to bring some seagods back to his palace for him to try. After eating it for a month, the emperor felt very vigorous and said the seagod worked wonderfully, but the name was not proper – “the god” (神; shen) usually refers to an immortal, and the homonym “ginseng” (参; shen) seems more proper. Since then, the seagod (海神; Haishen) has been called sea ginseng (海参; Haishen).

1.1.3 DRIFTINGS OF A FISHERMAN

Long, long ago, a fisherman was fishing in a blue sea on a fine day, and suddenly a cloud spread over the sea, and a strange wind rose. The fishing boat was blown off course for seven days and nights, until it was wrecked on an island. Then the cloud disappeared, the wind ceased, and the sea calmed down again. The fisherman, who was scared, hungry, and tired, struggled to survive. He looked around, but there were only trees, seabirds, reefs, and beaches. His experience told him that where there is a tree, there is fresh water. But, was there any food? Survival depends not only on water, but also on food. The sea was the obvious place for the fisherman to find food. Fortunately, the matches were not lost, which consoled the fisherman a little.

The fishing gear was lost at sea, and as the saying goes, if you have no hand, you cannot make a fist. In the warm sun and cool breeze, the fisherman tried to fish with his bare hands throughout the morning, but got nothing. “Am I going to die here?”, the fisherman wondered in despair. He tried again in the afternoon, and finally he got several strange organisms. “What are these?” The skillful fisherman with the experience of six generations of fishing ancestors was puzzled by the ugly sea worms. But hunger is the best sauce! The fisherman decided to trust destiny and cooked the organisms to eat them. When the fisherman woke up the next morning, he suddenly felt that he was no longer a castaway about to die. Instead, he felt vigorous, had rosy cheeks, got stronger, and felt he was 10 years younger. The fisherman thought, “These sea worms are treasures from the sea god, and I am not going to die.” After

eating the "sea worms" for several days, the old diseases caused by several decades of hardship fully disappeared. With his swimming ability, the fisherman got many such sea worms every day. However, the clever fisherman soon began to ponder how to preserve food for the coming winter. Due to the nutritious value of the sea worms, the fisherman had become strong and full of confidence. He cut wood and built a fine red house on the small island. He found that if sea worms were cooked, it would be very easy to dry them on the beach, and thus it would be easy to store them to be used later during the winter. However, the fisherman also wondered when he would be able to leave the island, and be able to tell his fellow villagers about the treasure from the sea god he had found, to also help them to become stronger and free from diseases.

After some time, the red house was discovered by a fleet of ships, and the sea worm's exceptional properties were rapidly disclosed to many people. As with the fisherman, after they ate this creature, they also became strong, vigorous, free of disease, and very fertile. Because the potency and efficacy of the magical sea worm resembled that of wild ginseng from the Changbai Mountain and Korea, it was naturally called sea ginseng.

1.2 NUTRITIONAL AND MEDICINAL VALUE OF SEA CUCUMBER

1.2.1 NUTRITIONAL VALUES

This section provides an overview; more details are available in Chapter 13 for tissue biochemistry and Chapter 19 for nutritional and medicinal values.

Sea cucumber contains a lot of water. The water content of *Apostichopus japonicus* is up to 91%, which is higher than pork, eggs, hairtails, prawns, and many conch species (Li et al., 2006). In the dried processed product, the protein level of *A. japonicus* is 34–60% (Li et al., 2006), and its total fat content is 3.5% (Li and Chang, 2006). The carbohydrate content of *A. japonicus* ranges from 2.1 to 3.8%, while that of other sea cucumber species is below 3% (Li and Chang, 2006). Its ash content is relatively high, between 28 and 37% (Li et al., 2006).

The body wall is the main medicinal (edible) part of the sea cucumber, and consists mainly of epithelial and dermal connective tissues (see Chapter 4). Dermal connective tissue cells contain abundant collagenous fibers, proteoglycans, glycoproteins, and amorphous interstitial substances. There are also many tiny calcareous elements called ossicles (Fan, 2001). Protein (polypeptides) content in the dried body wall is up to 90%, lipids are about 4%, and polysaccharides (oligosaccharides) about 6% (Katzman and Jeanloz, 1970); a small amount of nucleic acids is found as well. The inorganic components are calcium, magnesium, iron, manganese, zinc, copper, molybdenum, selenium, and other trace elements (Wang, 1991).

Analyses have shown that the nutritional ingredients found in the sea cucumber muscle bands (attached to the body wall) are similar to those of fish – the main ingredient being protein. The part containing the most nutritional ingredients and active substances are the coelomic epithelium and internal glandular ducts in the dermal connective tissue of the body wall, rather than the muscle. One of the key methods for evaluating the quality of sea cucumber is to verify that the coelomic epithelium and internal glandular ducts are abundant and well developed. However, at present, sea cucumber processors in China often discard these elements (coined "lining" and "tendon" in popular language) and overlook the fact that they are the most nutritional/potent body parts. They contain much of the sea cucumber's acidic mucopolysaccharide, fucoidin, saponin, and holothurin, among other active substances. Discarding them is a waste and decreases the quality of the final product (Guan and Wang, 2009).

1.2.1.1 Protein and amino acid contents and composition

Sea cucumber proteins consist mainly of collagenous protein (Matsumura et al., 1973), which is comparable in composition and efficacy to some traditional Chinese medicines, like donkey-hide gelatin, turtle shell glue, and antler gelatin (Fan, 2001). Results have proved that animal glue and gelatin are rich in glycine and basic amino acid, which are the foundation for hematogenesis, nourish the blood (biosynthesis of heme and globin), and facilitate calcium absorption (Fan, 2001).

Some studies have shown that the body wall of the sea cucumber contains many types of active polypeptides. The pentapeptides that consist of leucine, proline, serine, and arginine (some amino acids are of D-structure) are extracted from the epithelial tissue of *A. japonicus* and possess anti-inflammatory and antitumor activity; they have a relative molecular mass of 568.1 Da. The synthetic form of the pentapeptide also exhibits consistent efficacy (Fan, 2001). In addition, extracted glycoprotein is confirmed to prevent the growth of animal transplantation tumor through immune mediation (Suh, 1999; Wu et al., 2000).

There are 18 known types of amino acids in *A. japonicus*. Such diversity is comparable to what can be found in chicken egg proteins. Among the 18 amino acids, glycine and glutamic acid are the most abundant (Li et al., 2006). The amino acid content of *A. japonicus* is higher than that of other sea cucumbers, like *Holothuria* spp. and *Cucumaria* spp. (Su et al., 2003). The quality of the product depends greatly on the amount and proportion of essential amino acids, and whether they offer the essential amino acid proportions most suitable for human digestion and absorption (Gao, 2008). The essential amino acid in the body wall of *A. japonicus* ranges from 30 to 40% of dry body wall (Xiang et al., 2006), which is close to that found in the scallops *Chlamys farreri* and *Argopecten irradians* (Wang, 2007).

The taste (sensation of flavor perceived in the mouth and throat on contact with a substance) of sea cucumber is largely based on its content and composition in amino acids, principally aspartic acid, glutamic acid, glycine, alanine, serine, and proline. The presence of aspartic and glutamic acids is the main reason for a delicate flavor, whereas glycine and alanine confer a sweetish taste, together with serine and proline (Zhang et al., 2000). A study on wild *A. japonicus* off Zhangzidao Island shows that the flavor amino acids account for 51% of total amino acids (Li et al., 2006). This proportion is comparable to that found in the scallops *C. farreri* and *A. irradians* (Wang, 2007), but lower than in the clam *Solen grandis* (81%) (Dai, 2002), a species regarded as particularly flavorsome in Chinese cuisine.

1.2.1.2 Fatty acid composition

Fatty acid content and composition is another key component in assessing the nutritive value of food. Saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids are essential for human growth and development. In addition, unsaturated fatty acids are also important nutrients and flavor precursors. Increasing the content of unsaturated fatty acids, especially of highly unsaturated fatty acids, has been a growing dietary trend in recent years (Gao, 2008).

The lipid composition of sea cucumber consists mainly of phospholipid, which accounts for 90% of the total fat content. Tricosadienoic acid [*cis*-14-tricosenoi acid, 23:1(n-9)] is a fatty acid isomer that exists only in holothurians. It accounts for 1.6~7.3% of the total fatty acids, while cholesterol content is about 1%, indicating that sea cucumber is a low-cholesterol animal source (Fan, 2001; Svetashev et al., 1991). Polyunsaturated fatty acid is the main fatty acid constituting *A. japonicus* phospholipid, and consists of 13 varieties of carbonic acids, namely 18-carbonic acid (diene, triene, and tetraene), 20-carbonic acid (triene, tetraene, and pentaene), and 22-carbonic acid (triene, pentaene, and hexaene).

Arachidonic acid, 20:4(*n*-6), is the main polyunsaturated fat found in tropical sea cucumbers, and EPA, 20:5(*n*-3), is that of sea cucumbers from temperate seas (Gao, 2008). In some sea cucumber species (freeze-dried product), these two essential fatty acids can account for over one-third of total fatty acids, and DHA, 22:6(*n*-3), accounts for 6% of the total fatty acid content (Svetashev et al., 1991). Unsaturated fatty acid content of *A. japonicus* is higher than its saturated fatty acid content. Polyunsaturated fatty acid content is relatively high and accounts for 35–36% of total fatty acids (Li et al., 2006; Xiang et al., 2006). Among polar lipids, another key substance is sea cucumber saponin (holothurin), which is presented in Chapter 19.

1.2.1.3 Polysaccharides

Polysaccharide is a main component of the body wall, accounting for up to 6% of the total organic matter of dried sea cucumber (Katzman and Jeanloz, 1970). Two main varieties of polysaccharide are found in this tissue (Yoshida et al., 1992): the first is holothurian glycosaminoglycan (HG) or holothurian mucopolysaccharide, and the other is holothurian fucan (HF). HG is known to inhibit tumor growth; relieve the damages caused by radiation; alleviate inflammation, thrombus, and blood coagulation; reduce viscosity of whole blood or blood plasma; lower blood fat; raise immunity; prevent AIDS/HIV and hepatitis/HBV; promote hematopoiesis functional rehabilitation; and prevent pre-senile dementia (Gao, 2008). Its functions in promoting blood circulation and lowering blood fat can thwart cardio-cerebrovascular diseases, and its role in promoting hematopoiesis is effective in the treatment of anemia (Li and Chang, 2006; Su et al., 2003).

In addition, the very high content and sulfation degree of holothurian polysaccharides make sea cucumber a unique animal food. In recent years, attention has been paid to various polyanions, and healthcare and medical treatment functions of various polyanions are overall assessed. Sea cucumber is considered a polyanion-rich food (Gao, 2008).

Compared with other traditional Chinese medicines, sea cucumber has an advantage in the content of polysaccharides (details can be found in Chapter 19). Its recognized and putative medicinal efficacy and value are outlined next.

1.2.2 MEDICINAL VALUES

1.2.2.1 Sea cucumber in traditional Chinese medicine

Sea cucumber is often referred to as "ginseng of the sea," and it is recorded to have many health-preserving functions (Guan and Wang, 2009) in many ancient Chinese medical books (details in Chapter 19). Sea cucumber is sweet and salty in flavor, and neutral by nature according to Chinese traditional medical theory. It regulates heart, kidney, and lung activities; promotes spermatogenesis; cures impotence; enriches blood; normalizes menstruation; and strengthens waist and legs (in the principles of Chinese medicine, the health of the kidney is based on the strength of the waist and legs) (Guan and Wang, 2009).

The Chinese Medicine Theory is the summary of thousands of years of clinical experience; in some cases, it is somewhat vague, and in most cases, its scientific basis remains to be elucidated.

1.2.2.2 Sea cucumber in modern medicine

Modern biochemistry studies have shown that sea cucumber contains many active substances, such as mucopolysaccharide acid, holothurian fucan, chondroitin sulfate, holothurin, and holothurian peptides (Guan and Wang, 2009).

A. japonicus is considered the "king of sea cucumber," and its extract is attributed various properties: antithrombus, antitumor, antipathogenic, antifatigue; it is also said to enhance wound healing and

immunity (Guan and Wang, 2009). Sea cucumbers, especially *A. japonicus*, have already been put to clinical use (Fan, 2001; Guan and Wang, 2009). Some English-language literature has also been citing sea cucumber as the "ginseng of the sea" (sea ginseng), indicating that the medicinal values of sea cucumber are gradually being recognized worldwide (Chen, 2003; Fan, 2001).

1.3 SEA CUCUMBER IN CHINESE DIET

China has a very long history of consuming sea cucumbers. The literature shows that the Chinese have been eating sea cucumbers as early as the Three Kingdoms Period (Table 1.1) (Guan and Wang, 2009). During the Southern Song Dynasty (Table 1.1), sea cucumbers from Wenzhou were already

Table 1.1 Chinese Dynasties

Dynasties		Time Period	
Three Emperors		? BC–30th century BC	
Five Sovereigns		30th century BC–21st century BC	
Xia Dynasty		21st century BC–16th century BC	
Shang Dynasty		17th century BC–11th century BC	
Zhou Dynasty	Western Zhou Dynasty	11th century BC–771 BC	
	Eastern Zhou Dynasty	Spring and Autumn	770–476 BC
		Warring States	476–221 BC
Qin Dynasty		221–207 BC In 221 BC, Ying Zheng became the First Emperor of China	
Han Dynasty	Western Han Dynasty	202 BC–24 AD	
	Eastern Han Dynasty	25–220 AD	
Three Kingdoms		220–280 AD	
Jin Dynasty	Western Jin Dynasty	266–316 AD	
	Eastern Jin Dynasty	317–420 AD	
Northern and Southern Dynasties		420–589 AD	
Sui Dynasty		581–619 AD	
Tang Dynasty		618–907 AD	
Five Dynasties and 10 Kingdoms		907–979 AD	
Song Dynasty	Northern Song Dynasty	960–1127 AD	
	Southern Song Dynasty	1127–1279 AD	
Yuan Dynasty		1271–1368 AD	
Ming Dynasty		1368–1644 AD	
Qing Dynasty		1636–1912 AD	

From Xu, 2000

well known, and had been transported to Lin'an as the top-grade rarity in the capital. Sea cucumbers from Zhejiang Province were once called Shaxun. Wenzhou people understood the ecological habit of Shaxun, learned how to catch and process them, and their skill reached a "unique and incomparable" level. Consumers reported that this sea cucumber species was "crispy outside, smooth and delicate inside, and tastes very good," and that fishermen deserved a lot of money for harvesting it. From the perspective of modern taxonomy, sea cucumbers from ancient Wenzhou should have been classified into Guangshen (Holothuriae, sea cucumbers without spike). People consumed the Guangshen species before the Cishen species (Stichopodidae, sea cucumber with spikes). However, Cishen (*A. japonicus*) eventually became the preferred species and was classified as a marine rarity (Wang, 2003).

When appraising a sea cucumber, the ancients paid more attention to its origin. Historians only recorded Cishen and Denglaishen, namely *A. japonicus* today, as the most famous and best-quality sea cucumbers.

The *Collection of Stories in Qing Dynasty Animals* reads, "It was once called 'Shaxun' and 'Haishen' (synonyms for sea cucumber) after being dehydrated. Today, fresh and dried forms are both commonly called Haishen ("Hai" means sea and "shen" as "seng" in ginseng, means tonic). The black thorny ones produced in Fengtian (now called Liaoning Province) are the best; they are known as Cishen (thorny Haishen) or Liaoshen, or Hongqishen. The region of Lüshun in Western Korea Bay is the northernmost area of the Yellow Sea and the Bohai Sea where sea cucumbers (*A. japonicus*) are produced in China. Before the government of the Qing Dynasty (Table 1.1) ceded the eastern area of the Wusuli River to Tsarist Russia, former Chinese marine territories under the governance of Jilin (now Peter the Great Bay in current Russia) also produced precious sea cucumbers (*A. japonicus*). Volume 7 of *Jilin Choreography* by Sa Ying'e mentions clearly, "The sea cucumber is of a worm shape and has flesh thorns. Those from Hunchun are the best." Hunchun was an important town in east Jilin Province in the Qing Dynasty. Haishenwai (now Russian Vladivostok, "Haishen" means the species and "wai" means a bay), a town administrated by Hunchun City in the Qing Dynasty, produced excellent sea cucumbers (*A. japonicus*). The name of "Haishenwai" refers to the location where *A. japonicus* was produced (Wang, 2003).

Cishen from Shandong were called Denglaishen in ancient times, and were also considered to be of excellent quality. The *Records on Marine Fish Resources* by Hao Yixing reads, "There is an organism about 1 *chi* (1 *chi* = 1/3 m) long in the Denglai Sea (present-day Shandong Province). This organism is light yellow, its body consists of meat without bone, it has no mouth, but has intestines and stomach… It is called Haishen (*A. japonicus*)." The *Mouping County Annal* also reads, "The sea cucumbers from the sea area of Yangma Island (an island in Yellow Sea) are the best."

In ancient China, the culinary culture in a royal family represented the highest level of this art. *The Book of Songs* reads, "Every inch of land belongs to the Emperor, and all people are under the control of the Emperor." Hence, the food offered at the imperial court was excessively luxurious. It was prepared with the finest raw materials and under the best conditions at the time, following the principle of being well presented, delicious, healthy, life prolonging, and honorable. Incomparably fine food was created, exemplifying the scientific level and cultural features of Chinese culinary art. In addition to the splendid, elegant, dignified, luxurious, honorable, and fine diets of ancient Chinese emperors, solemn dining ceremonies, grand spectacles, harmonious appearances, and the high quality of food are also shown. Therefore, culinary activities were an exercise of physical, mental, as well as scientific and artistic expression (Zhao, 2003).

Food used by the imperial courts must therefore have been of the highest grade found in the world. The sea cucumber *A. japonicus* was perceived as the greatest tribute offered by local high officials in Shandong and Liaodong peninsulas to honor the emperor, imperial concubines, or senior officials at court (Liu, 2012). According to the *Royal Court History of Ming Dynasty*, "Xizong Emperor likes to combine over 10 kinds of fine foods together to serve, including roasted clams, fresh shrimps, bird's nest, shark fins, sea cucumber ..." (Xue, 2009).

The imperial court could employ and command any famous chef and collect any food in the world. Cumulating knowledge from several dynasties, the Chinese culinary art reached its peak in the Qing Dynasty (1644–1912) (Zhao, 2003). Of particular note was the world-renowned Man-Han imperial feast, a full, formal banquet, combining Manchurian and Han delicacies of the Qing Dynasty. On the menu passed down to the present, sea cucumber is present in 12 of the 217 dishes. The famous sea cucumber (*A. japonicus*) dishes in Shandong cuisines – "Family Portrait" – was named by Emperor Qianlong in person (Xue, 2009). The latter-day "Sea Cucumber Banquet" also prevailed at that time. All these indicate the value attributed to *A. japonicus* by ancient Chinese emperors and ruling classes.

Although sea cucumber was ranked first among the "Eight Marine Delicacies," it was mostly enjoyed by the imperial family because it was very rare and expensive. The *Records on Changdao Sea Cucumber Restaurant* by Ji Xiaolan reads, "Sea cucumbers (Cishen) are tributes to court in all dynasties, and are high-end foods. Common people can hardly ever taste them."

As societies continue to develop, more and more people tend to advocate "green," "natural," and "safe" dietary concepts. Sanitation, nutrition, science, and food safety have become major social foci with advocates across social scales, including government authorities, academic and educational institutes, food industries, restaurants, consumers, and the media. This progressive, green, and scientifically based dietary concept has gradually been accepted by modern Chinese people (Zhao, 2003).

Sea cucumbers today are widely accepted by Chinese people because they meet the dietary cultural concept of modern people – not only for satiation and delicious taste, but also for health.

Thanks to a joint acceptation by traditional Chinese medicine and modern science, sea cucumber has become a high-end health food in modern China. As industrialization of sea cucumber culture, processing, and marketing progresses, sea cucumbers will gradually become affordable; and as the saying goes, "The swallow used to inhabiting only under the nobles' eave flies into common people's house."

1.4 CULTURAL EXPRESSION

Sea cucumber was discovered over 1000 years ago in China. It has since then permeated the life and culture of Chinese people, and has also woven its way into Chinese art.

1.4.1 CALLIGRAPHY OF "参" (GINSENG AND SEA CUCUMBER)

Chinese calligraphy is the unique artistic form of Chinese characters; it can be traced back to inscriptions on bones or tortoise shells during the Shang Dynasty (second millennium BC; Table 1.1). The character "参" is shared by ginseng and sea cucumber (i.e., ginseng of the sea). The characters inscribed on the oracle bones numbered as 17599, 17600, and 17601 over 3500 years ago are the earliest known ancient "参" characters (Figure 1.2). These ancient characters present the shape features of the Araliaceae ginseng. The upper part of the character represents the ginseng fruit with a pedicle, and

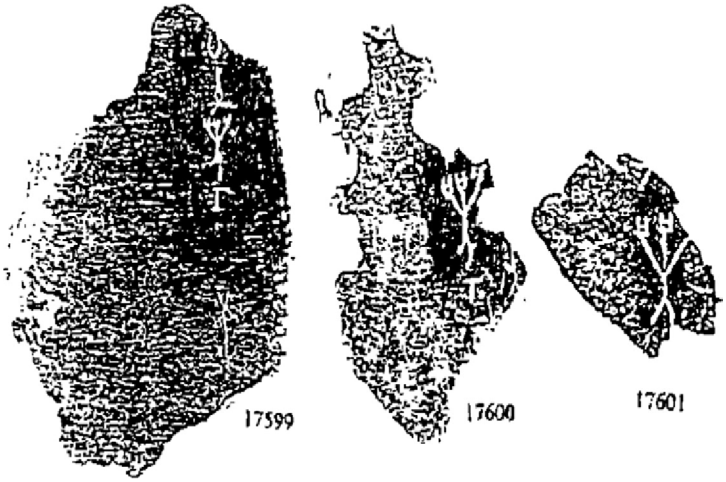

FIGURE 1.2

The character "参," which represents the plant named ginseng and is also used to represent the sea cucumber (ginseng of the sea), was already in use during the Shang Dynasty. Here, it is shown engraved on oracle bones, numbered 17599, 17600, and 17601, dating back more than 3500 years. Oracle bone inscriptions are the origin of modern Chinese characters.

Zou, 2000

the lower part represents the herringbone axial root and rootlets (Zou, 2000). In Chinese medicine, ginseng is a very famous and noble element with very high value and efficacy. It is called "the king of all Chinese herbal medicines." A famous Chinese ancient medical book entitled *Shen Nong's Herbal Classic* reads, "Ginseng can tonify the five organs (heart, liver, spleen, lungs, and kidneys), soothe the nerves, stop palpitation, remove evil factors, improve acuity of sight, and promote intelligence"; "usage of ginseng for a long period can increase longevity, dexterity, and agility." In the *Compendium of Materia Medica* (Figure 1.3), the author Li Shizhen also thought very highly of ginseng. He believed ginseng could "cure all deficiencies in men and women." Over several thousands of years, ginseng has always ranked as the top product in Chinese herbal medicines. The nutritional and medicinal values of sea cucumber are believed to equal those of the ginseng plant (see Chapters 13 and 19), hence its name and the shared calligraphy.

In ancient Chinese, only one character was typically used to describe something. Therefore, "参" could refer to ginseng, sea cucumber, or more specifically to *A. japonicus*, depending on the context. The bisyllablization marked a change in the Chinese language. Today, the use of two characters makes it easier to distinguish "人参" for ginseng, "海参" for sea cucumber, and "刺参" for *A. japonicus*. As the symbol of sea cucumber (ginseng as well), the character "参" frequently appeared in calligraphy art, the core of Chinese traditional culture.

The characters "参" listed in Table 1.2 illustrate the evolution of Chinese character calligraphy, from irregular lines to regular strokes. The earliest calligraphy style is the seal script, including large-seal and small-seal scripts. Large-seal script consists of inscriptions on bones or tortoise shells in the Shang Dynasty (Table 1.1), inscriptions on ancient bronze objects, Zhouwen (a style of calligraphy

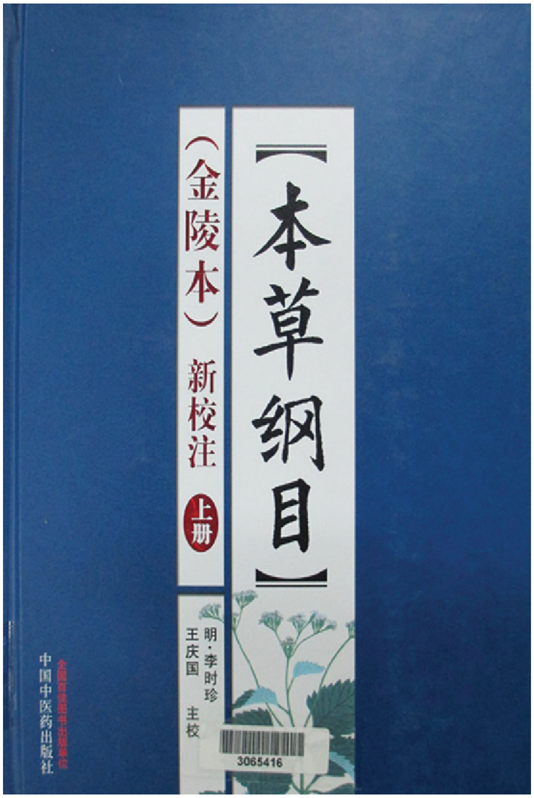

FIGURE 1.3

The book *Compendium of Materia Medica*, by the author Li Shizhen (1518–1593).

current during the Zhou Dynasty), and six state scripts. The obvious features of ancient pictographic characters are kept in the large-seal script. In the Qin Dynasty (Table 1.1), prime minister Li Si organized some civil officers to standardize the small-seal script, and confirmed the small-seal script as the official characters around the country. Therefore, the small-seal script is also called the "Qin-Dynasty Seal Script."

In the Western Han Dynasty (Table 1.1), the Chinese clerical script evolved from the seal script, which marks a significant progress in the evolution of Chinese writing and calligraphy. From then on, Chinese characters tended to be upright and square, and more techniques of writing were introduced, which broke the single technique of the seal script and laid a foundation for various scripts afterward. The sentence "Since the Later Han Dynasty, stone tablets became a fashion" indicates the maturity of the clerical script in the Han Dynasty.

In the Han Dynasty, the cursive script was used alongside the clerical script. The occurrence of the cursive script indicates that calligraphy began to become an art that can express the feelings and personality of the calligrapher in a free manner.

Table 1.2 Character "参" in Different Calligraphic Styles

 The seal script 1 the *Origin of Chinese Characters* (in 121)	 The seal script 2 Qing Dynasty-Deng Shiru (1743–1805)	 The seal script 3 Qing Dynasty-Xu Sangeng (1826–1890)	 The seal script 4 Qing Dynasty-Wu Dacheng (1835–1902)
 The clerical script 1 Han Dynasty-Shi Chenbei (in 169)	 The clerical script 2 Han Dynasty-Cao Quanbei (in 185)	 The clerical script 3 Mawangdui Silk Texts dug out of earth in 1973	 The clerical script 4 Mawangdui Silk Texts dug out of earth in 1973
 The regular script 1 Tang Dynasty-Chu Suiliang (596–658)	 The regular script 2 Tang Dynasty-Ouyang Xun, inscriptions on Liquan Spring in Jiucheng Palace (632)	 The regular script 3 Tang Dynasty-Yan Zhenqing (709–784)	 The regular script 4 Tang Dynasty-Liu Gongquan (778–865)

Table 1.2 Character "参" in Different Calligraphic Styles *(cont.)*

 The running script 1 Eastern Jin Dynasty-Wang Xizhi (303/321–361/379)	 The running script 2 Eastern Jin Dynasty-Wang Xianzhi (344–386)	 The running script 3 Tang Dynasty- *Jinci Temple Inscriptions and Preface* by Li Shimin (599–649)	 The running script 4 Song Dynasty-Mi Fu (1051–1107)
 The cursive script 1 Eastern Jin Dynasty-Wang Xizhi (303/321–361/379)	 The cursive script 2 Eastern Jin Dynasty-Wang Xizhi (303/321–361/379)	 The cursive script 3 Tang Dynasty-Sun Guoting (646–691)	 The cursive script 4 Mao Zedong (1893–1976)

In Chinese, "参" refers to ginseng, as well as sea cucumber (ginseng of the sea). Each row presents a different style. All five styles, even the earliest seal script, have always been used but were fashionable to different degrees in the various eras. Therefore, though the styles appeared chronologically, the works using the different styles are not in chronological order, for example, the seal script appeared the earliest (about 3000 years ago), but it became popular as calligraphy art much later, in the Qing Dynasty (1636–1912 AD).

Wu, 2001

In the Three Kingdoms Period (Table 1.1), the regular script created by Zhong Yao began to become mainstream. The representative works of the regular script are the *Remonstrance for Recommending Ji Zhi*, and the *Remonstrance for Accepting Sun Quan as an Official* in tablet inscriptions of the Northern Dynasties.

In the Western and Eastern Jin dynasties (Table 1.1), the running script excelled with a splendid achievement in calligraphy. The running script is a transitional script between regular script and cursive script. When mentioning the Western and Eastern Jin dynasties, we must not leave out the two

calligraphy masters, Wang Xizhi and Wang Xianzhi, a father and his son. Wang Xizhi, the father, is called the "calligraphy god," and his *Preface of Orchid Pavilion Collection* is entitled "the best running script in the world," with reviews like "as light as a floating cloud, or as powerful as a surprised flying dragon." The *Ode to Godness of Luoshui River* by Wang Xianzhi, the son, created a powerful style in handwriting, and his "poti" ("po" — to break, "ti" — style; "poti" means styleless or without a constraint in style) and "one-stroke handwriting" contributed greatly to Chinese calligraphy in history.

In the Northern and Southern dynasties (Table 1.1) when the country was in turmoil, calligraphy consisted of the stone inscriptions in the Northern Dynasty and handwriting models in the Southern Dynasty. In the Sui Dynasty (Table 1.1), the stone inscriptions and handwriting models were combined, which formally completed the regular script. This forms a link between the past and recent calligraphy histories.

Calligraphy reached its peak era at the beginning of the Tang Dynasty (Table 1.1). The Tang Dynasty was a period of great prosperity, being the heyday of Chinese feudal culture, and the peak of Chinese calligraphy as well.

There were many very brilliant and famous calligraphers in the Tang Dynasty. Ouyang Xun was a master of the regular script at the beginning of the Tang Dynasty. Zhang Xu and Huai Su, jointly known as "Lunatic Zhang and Drunken Su," were two masters of the wild cursive script in the flourishing period of the Tang Dynasty. In the Middle Tang Dynasty, Yan Zhenqing set a standard for the regular script, and this standard became the orthodox standard. In the Late Tang Dynasty and the Five Dynasties, Liu Gongquan developed the regular script, making use of a "strong but powerful" style, which further enriched it.

In the Tang Dynasty, the five kinds of Chinese calligraphies, "the seal, clerical, cursive, regular, and running scripts" had been fully confirmed. Calligraphy continued developing, and many calligraphy masters arose, like the four masters in the Song Dynasty (Table 1.1), "Su, Huang, Mi, and Cai" (Sushi, Huang Tingjian, Mi Fu, and Cai Xiang). Zhao Mengfu in the Yuan Dynasty (Table 1.1) created a new style of the regular script "Calligraphy style of Zhao Mengfu," which is jointly known as "the four calligraphy styles" with the "Tang regular script calligraphy styles of Ouyang Xun, Yan Zhenqing, and Liu Gongquan."

The characters "参" listed in Table 1.2 were written by famous calligraphers. From these handwritten characters, we can see the elegant aspect and charm of Chinese calligraphy. The frequent appearance of "参" in calligraphy, especially in the famous works, also indicate that the sea cucumber has always been an important part of the life of the Chinese.

1.4.2 POEMS AND ESSAYS ON SEA CUCUMBER

Sea cucumber is so important in Chinese tradition and history that it has been the subject of numerous texts, highlighting its significance in the daily life of the people and showing deep roots in imperial customs (shown previously).

1.4.2.1 Ancient poems

In the long Chinese literary history, ancient literature sometimes features the sea cucumber. All translations of poems in this section, except "*Autotomy*", are by the authors of this chapter. (Admittedly, some of their beauty has probably been lost in translation).

海参 (*Sea Cucumber* by Wu Weiye, a famous poet in the Qing Dynasty)

预使燂汤洗，迟才入鼎铛。

禁犹宽北海，馔可佐南烹。

莫辨虫鱼族，休疑草木名。

但将滋味补，勿药养余生。

Translation:

Clean sea cucumbers with warm water, and cook in a jug.
Best produced in the north as royal tribute, and served with food in the south.
It does not matter whether it is animal or plant.
It by all means could nourish you without any drug.

海参赋 (*Sea Cucumber Ode*) (author unknown) also describes the sea cucumber:

东溟千里，海错缤纷，其中宝物，名曰海参。庖宰视之为三绝，食界列之于八珍。倘若高厨临灶，大师掌门，必使菜色夺目，香气迸喷。实可谓出鼎鼒而色味动客，入脾胃而营养宜人。今有速健海参王者，技绝艺真，在此操饪，尽展芳芬。于是酒徒群集，食客盈门，八方相告，远近传闻。知者不惜千金而求座，不知者亦能一尝而惊唇。乃敢说北国有店，入化出神，海参烹调，惟此独尊。

Translation:

In the boundless Eastern Chinese Sea, there are various marine organisms. A valuable one is called sea cucumber. Cooks deem it as one of the “three top delicacies,” and gastronomes rank it as one of the “eight top delicacies.” If a master chef cooks it, the dish will look dazzling and taste delicious; guests are attracted by its delicate appearance and its nutritious properties. Today, a master chef referred to as “sea cucumber cooking king” came here to show his skill. Then, many drinkers and customers came along, and people told each other about the delicacy. Therefore, the delicacy became well known. The people who heard about the delicacy paid a lot to taste it, and those who had never tasted it were surprised by the taste. People believed that a northern restaurant can provide magically delicious food, especially sea cucumber.

Xu Jizhi, who lived during the Southern Song Dynasty in Wenzhou, wrote a poem to praise the sea cucumber. The *Informal Thank-you Essay for Shaxun Given by De Jiu*, Volume 4 of the *Collection of Works of She Zhai*, reads:

沙噀噀沙巧藏身，伸缩自如故纳新。

穴居浮沫儿童识，探取累累如有神。

钧之并海无所闻，吾乡专美独擅群。

外脆中膏美无度，调之滑甘至芳辛。

年来都下为鲜囿，独此相忘最云久。

转庵何自得此奇，惠我百辈急呼酒。

人生有欲被舌瞒，齿亦有好难具论。

忻兹脆美一饷许，忏悔未已滋念根。

拟问转庵所从得，访寻不惜百金直。

岂非近悟圣化时，望慈尤物令人识。

Translation:

Shaxun (ancient formal name for sea cucumber) hides smartly in sand, and moves freely.
Even children could recognize the crevasses where Shaxun hide and can get many with the help of a god.
No sea cucumber is known to exist in other seas; only our hometown has valuable ones and our home folks can cook them incomparably.
Crispy outside, smooth and delicious inside, they taste fantastic!
The unique and delicious flavor lingers for a long time.
Lucky to get the chance to appreciate the delicacy, I hastily order wine to favor not only my tongue, but also my teeth.
Enjoyed this crispy delicacy for only a moment, I regret not having dug out the origin of this animal.
I ask how the host got the sea cucumber, and why people wish to pay a lot of money for it.
It is a great honor for me to understand a bit more about the mysterious animal.

1.4.2.2 Modern literature

There are many excellent works in modern literature that continue to praise sea cucumber. Wei Yong (2012) has written a poem entitled 海参 (Sea Cucumber):

海参将逃亡后损半的身体复原如一。

必须要赞叹，这生命的伟力

必须要赞叹，寂静地忍耐和虔敬地希望。

正如面对死亡，抛弃内脏，

抛弃将半喘息承受的疼痛，只有疼痛了才知道

此时的承受，就是彼时的幸福，

生命有唯一的道路。要平静地面对

发生的一切，要用冷默的眼神展望世界，

要用淡定的心理解，发生的

生命和未来的生命，就是生命的必须历经。

大声地呼喊永远屈服于寂静地期盼，

对将来的期盼才是力量的捕获，

过去就是死亡。正如这首小诗的渴望，

必须继续，但是要停止，但是

远还没到停止之时，一切的痛苦不过是

痛苦中的痛苦，疼痛亦是如此，

一切都存在有如一的幸福，即使零的虚无

晃荡在以大的天穹，即使抛弃了

一的部分，依然要复生一，依然要忍耐和等待

依然要希望，才可以进入到生命意义的本身。

沉浮于动荡的水中或飘行于寂静的水上，思想剥离掉三重伪装

轻盈，闪光，以及遗忘。

Translation:

Must recover and survive!
A sea cucumber did after a big body loss.
What a great power, a strong life!
Silent fortitude is the only way to success.
Death coming, it discards the guts!
Bear unbearable pain and realize
this pain brings about fortunes.
Life is a one-way journey.
Stay calmly, one who can stay wins!
Looking at the world indifferently,
Today and tomorrow are all life tracks.
To yell rather than to make a quiet wish,
Grasp the power in persistency.
The past dies! Life journey relays
as this poem wish, with rest and pause.
Still, a long, long way to the death,
All suffering is just a sore in pain.
Everything may breed happiness!
Nothingness ether in the sky dome pervades,
and the integrity no longer exists,
it must recover, tolerate, and wait, regardless!
Staying in hope can taste the life essence.
Dancing in running or on still water,
Stripping off heavy camouflage in mind,
One becomes swift, glorious, and oblivious!

The representative work of the writer Deng Gang – a long novel called *White Sea Cucumber* – describes the "personal world" of Ma Li, a 17-year-old skillful "fisher" living by the Sea in Dalian, from his own perspective. In times of turmoil, the pursuit of the "white sea cucumber," fantastic friendship and love, makes the white sea cucumber mysterious.

Besides these literature masters, common people also wrote about and praised the sea cucumber. Like the poem 海底八珍——七律·海参 (2012) (*One of Eight Sea Treasures— Sea Cucumber*) from "Poem Column of China Literature Forum."

前世修行卧玉床，刺裘护体面羞光。

时时寻觅沙中宝，日日恭承海底王。

寸步途程挪几岁，尽心悟道历三霜。

忽如一日仙班列，浴火重生披锦装。

Translation:

Lay on a jade seabed as a result of evolution, with a thorny coat for protection.
Search for the treasure in the sand from time to time and pay daily respect to the King of the Ocean.
Move forward inch by inch for the truth of a lifetime in every season
All of a sudden die as an immortal, and be reborn in brocade fashion.

And the original poem 白海参之梦 (2012) (*Dream of White Sea Cucumber*), posted on NetEase blog, "sea-Friederike" by Yan Dong:

就要化为乌有的时候

退却的潮汐还在远远地招手

默然回首

漂浮的夕阳也似无水可依

西沉的归路早已没了轻舟

那一片红红的霞缕

是海滩之上

最后的温柔

你就这样搁浅

置身于波涌之外

却无意祈求波涌的滞留

你就这样安然

洁身而去

宁肯一点一滴的耗尽

柔媚的灵骨

你美丽地弥散

伴着依旧涛声

你无声地氤氲

却消隐得自由

将仅存的意识付诸东流

为的却是完结与水的

一生厮守

不在猎取者的手中

便有千万种生存的理由

注：白海参为海中稀有之物。传说，此珍贵之物一旦被潮汐弃之海滩，便自融为液，沉于沙中。因而，渔人只见过白海参，却从未得之。

Translation:

When the sea cucumber is about to vanish,
The falling tide is still waving remotely,
Billowing the empty time and tide.
Turn round silently,
The setting sun lost support at the water surface,
There is no boat on the way back home,
The slice of rosy clouds,
Scatter final softness

Onto the beach.
You are stranded in this way,
Beyond waves
Not praying for waves to stay
You leave quietly
And cleanly
Exhaust your soft body
Little by little
You disperse yourself beautifully,
Accompanied by familiar wave sounds
You dissipate soundlessly,
And disappear freely,
With only consciousness left behind
To continue the eternal life staying together with the sea
You have thousands of reasons to survive
So long as you are not caught by a hunter.

Note: The white sea cucumber is rare in the sea. It is said that once this valuable organism is discarded on the beach by the tide, it dissolves into liquid, and penetrates into the sand. This is why fishers can see white sea cucumber but cannot get it.

In addition, the autotomy and regeneration of the sea cucumber deeply touched Wislawa Szymborska. So she created the poem *Autotomy* in 1972:

In danger, the holothurian cuts itself in two:
It abandons one half to a hungry world
and with the other half of itself flees.

It violently divides into doom and salvation,
retribution and reward, what has been and what will be.

An abyss appears in the middle of its body
between what instantly becomes two foreign shores.

Life on one shore, death on the other.
Here hope and there despair.

If there are scales, the pans don't move.
If there is justice, this is it.

To die just as required, without excess.
To grow back just what's needed from what's left.

We, too, can divide ourselves, it's true.
But only into flesh and a broken whisper.
Into flesh and poetry.

The throat on one side, laughter on the other,
quiet, quickly dying out.

Here the heavy heart, there *non omnis moriar*,
just three little words, like a flight's three feathers.

The abyss doesn't divide us.
The abyss surrounds us.

(In memory of Halina Poswiatowska)

Comments by Li Yanxia: Wisława Szymborska is the 1996 recipient of the Nobel Prize in literature. She discloses the historical background and ecological laws in many aspects of real human life through accurate allegories. We can see ins and outs through her poem named *Autotomy* – in danger, the holothurian cuts itself in two: it abandons itself to a hungry world and with the other half of itself flees. She began the poem with the autotomy of the sea cucumber helping itself in danger, further dug out the meaning of autotomy, and dedicated it to the life, world, mental well-being, and spirit, step by step. What a powerful poem!

How time flies! The sea cucumber has been known for over 1000 years. Talking about sea cucumber is just like reading Chinese history. During this long historical period, sea cucumber has been deeply branded by the Chinese. It is all at once a potent drug in traditional Chinese medicine, a delicacy in Chinese cuisine, a favored gift and tribute, and an object of art. It is intimately linked with the life of Chinese people. What is more important is that it is just like a mysterious and beautiful natural angel that brings health and frailty to human beings, and drives people to explore knowledge and tenaciously probe into it. Keeping with tradition and history, and heartfelt ancestral ties to sea cucumbers, Chinese scholars are still studying the legendary species today. This ancient life form, over 0.6 billion years old, is now crossing paths with human beings, but has yet to reveal all of its secrets. All the more reason to explore and study it!

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CHAPTER

FROM FISHERIES TOWARD AQUACULTURE

2

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SUMMARY

In China, *Apostichopus japonicus* has a long history of consumption, owing mainly to the health benefits and medicinal properties attributed to sea cucumber. The traditional dive-fishing method for *A. japonicus* is still being used in China today. With the socioeconomic boom in the 1990s, the market demand for sea cucumber increased considerably and led to the sharp decline of natural resources. Consequently, *A. japonicus* aquaculture emerged and became the most important source of *A. japonicus* on the market. With increasing wealth and consumer demand in China, *A. japonicus* aquaculture is expected to expand in years to come, despite limiting factors, such as diseases, environmental deterioration, and space constraints.

Keywords: aquaculture development; *Apostichopus japonicus*; fishing history; limitations; sea cucumber; trends

2.1 THE HISTORY OF *A. japonicus* FISHERY

2.1.1 EARLY MENTIONS AND FISHERY

The earliest records of sea cucumbers appear in a book called 临海异物志 (*Adversaria of Monster by Sea-shore*) (published between the years 268 and 280), which was written by Shen Ying during the period of the Three Kingdoms (see Table 1.1 in Chapter 1). "The meat looks like black soil, the diameter of a child's arm, about five cubits long, with the abdomen in the middle and thirty tube feet around the body, without mouth and eyes, and it can be cooked." The black soil meat refers to sea cucumber. In 江赋 (*The Ode to Rivers*) written by Guo Pu (276–324) during the Eastern Jin Dynasty, it was on a par with other prized food, such as pen shell meat. Sea cucumber was rarely referred to for a long period of time after that, until it was noted again by Jia Ming in 饮食须知 (*Preliminary Instructions for Food*) (ca. 1367) during the Yuan Dynasty: "Sea cucumber tastes sweet and salty." More records emerged subsequently, such as in 药鉴 (*Drug Discrimination*) (by Du Wenxie during the Ming Dynasty, ca. 1598), 五杂俎 (*Five Miscellaneous*) (by Xie Zhaozhe during the Ming Dynasty, ca. 1616), 闽小记 (*Fujian Note*) (by Zhou Lianggong during the Ming Dynasty, ca. 1647–1672), and 本草从新 (*New Compilation of Materia Medica*) (by Wu Yiluo during the Qing Dynasty, ca. 1757). Those documents narrate the habitat, appearance, and restorative function of sea cucumber, without any references to fisheries. In 1765, 本草纲目拾遗 (*A Supplement to Compendium of Materia Medica*) (by Zhao Xuemin during the Qing Dynasty) noted the harvesting of sea cucumber in Guandong, Penglai, Fushan, and mentioned the time, process, and skill required to fish them. According to the ecological characteristics and

Liu, G., Sun, J., Liu, S. 2015. Chapter 2. From fisheries toward aquaculture. In: Yang, H., Hamel, J.-F., Mercier, A. (Eds.), The Sea Cucumber *Apostichopus japonicus*. History, Biology and Aquaculture, Academic Press, pp. 25–36. http://dx.doi.org/10.1016/B978-0-12-799953-1.00002-7

geographical distribution of different species of sea cucumber in China (Liao, 1997), the sea cucumber mentioned in the latter book is *A. japonicus*. We can therefore conclude that a dedicated fishery for *A. japonicus* was established no later than the middle of the eighteenth century. See Chapters 1 and 20 for more details on the long-standing cultural and culinary traditions surrounding sea cucumber in China.

2.1.2 FISHING TECHNIQUES

In the book *A Supplement to Compendium of Materia Medica*, it is recorded that Han Ziya in Guandong said “once they find the sea cucumbers, they dive in the water to capture them ... divers explore and catch sea cucumbers by hand, placing them around their neck, under their armpits, or between their legs,” and Li Jinshi of Penglai records said “sea cucumber also can be found in shallow sea of Dengzhou, ... mostly crawling on big stones, in areas too deep to be captured. The people spread seal oil on the surface of the water, when it disperses, the water surface becomes clear enough to see through it to localize and catch sea cucumber. The harvesters are attacked by sharks constantly, thus increases the value and cost of sea cucumbers.” It is also recorded that Chen Lianghan of Fushan said “the sea cucumbers lurk on the bottom of the ocean in March and April ... reproducing and therefore easy to capture; ... It is difficult to catch sea cucumbers when they move to the deep and attach firmly to rock in May and June; … During canicular days, the sea cucumbers hide under rocks or in the mud in deeper areas and are harder to harvest; ... During hibernation, sea cucumbers migrate to the bottom of the deep sea and cannot be caught.”

The traditional dive-fishing method for *A. japonicus* is still being used in northern China, the only difference being that the diver's equipment and the containers for storing the sea cucumbers have been improved (Zhang and Wu, 1955). In general, divers leave the boat, seek *A. japonicus* visually between 2 and 20 m depth, and then collect them by hand and store them in mesh bags tied to their waist. The bags are made of nylon or polypropylene rope, with a mesh size of 0.5–1.0 cm, a length of 60–80 cm, an opening diameter of 15–20 cm, and a bottom diameter of 40–50 cm. The divers work underwater for about 1–2 h, less in winter when lower water temperatures prevail. At the same time, workmates on the boat monitor the air supply, and sort the harvest that divers bring back at regular intervals (Figure 2.1). There are currently two different operating methods for harvesting *A. japonicus* in China: (1) the so-called hookah method, which involves an airline fed by a compressor, providing unlimited air supply but allowing a limited range of activities for divers; (2) free diving, allowing free movements, but limited time underwater.

The sea cucumber fishery is a traditional industry operated by coastal fishermen in China. As far as 1681, fishermen went around to the Xisha and Nansha islands fishing grounds for sea cucumbers, such as *Thelenota ananas* and *Holothuria nobilis*; since then, fishery has never stopped, according to historical records. Sea cucumber fishing in the early days, mainly depending on free diving, was inefficient, and did not allow to harvest beyond 20 m depth; it did not involve large sea cucumber specimens found mainly in deep water. The cucumber-fork was invented in the 1920s to collect cucumber species in the Nanhai Sea (Li, 1990). There is no similar fishing tool used to collect *A. japonicus* in historical records; people still harvest *A. japonicus* by hand in China.

2.2 AQUACULTURE DEVELOPMENT

It has been about a thousand years since people started to explore and utilize *A. japonicus* in China (see Chapter 1). However, the aquaculture of *A. japonicus* emerged only in the past century. Early as 1954, Zhang Fengying and his collaborators tried to culture *A. japonicus* on an artificial reef in

FIGURE 2.1

The traditional dive-fishing method for harvesting *A. japonicus* in northern China. (A) Diver leaving the boat in search of sea cucumbers, using a hookah as air supply. (B) Diver bringing back a bag full of *A. japonicus*. (C) Technicians sorting the harvest aboard the boat. (D) A sample of *A. japonicus* collected at about 10 m depth.

Photos by Guangbin Liu

Beidaihe, Hebei Province (China) and obtained good results. Due to the poor living standard at that time, the nutritive value of sea cucumber was not widely recognized and market demand was limited. Natural resources of *A. japonicus* were abundant and could easily satisfy market needs, precluding the need for any aquaculture industry to develop. With the socioeconomic boom in the 1990s and the ever-growing evidence of health benefits associated with the consumption of *A. japonicus*, the market demand increased significantly, which led to excessive harvest of wild *A. japonicus*. It became almost extinct in several areas of its native distribution range during the same period. In the 1960s, *A. japonicus* fishing output was 260–280 t in Shandong and Liaoning Provinces, but it sharply dropped to 60–80 t by the 1970s (Zhang and Liu, 1984). As the natural stocks of *A. japonicus* could no longer satisfy the growing market, *A. japonicus* aquaculture emerged.

2.2.1 ARTIFICIAL PRODUCTION OF SEEDLINGS

In 1953, Zhang Fengying, from the Institute of Oceanology, Chinese Academy of Sciences, in collaboration with the Fisheries Research Institute of Hebei Province, conducted the first research project on the breeding of *A. japonicus* and obtained the first artificially bred juveniles of the species (Zhang et al., 1958). In the mid-1980s, the Fisheries Research departments in Hebei, Liaoning, and Shandong provinces established an industrial breeding program for *A. japonicus* with yields reaching more than 3×10^3 ind. m^{-2} (for juveniles of ~5 mm) (Liao, 1986; Mou et al., 2000). With developments and improvements made over the following decade, the technology for the artificial breeding of *A. japonicus* matured, greatly promoting the rapid development of the aquaculture industry in the mid-1990s. In 2009, the total production of *A. japonicus* seed in China was 5.18×10^{10} ind., including 3.02×10^{10} ind. in Shandong Province and 2.05×10^{10} ind. in Liaoning Province.

Artificial breeding of *A. japonicus* for the production of seedlings includes the following stages (refer to Chapter 16 for additional details on aquaculture methods):

1. Collection and maintenance of broodstock. *A. japonicus* adults are gametogenically mature between the end of June and the end of July, with slight shifts based on local seawater temperature. The broodstock collected by divers is maintained in culture ponds. The weight of adult broodstock of *A. japonicus* should be above 200 g and the cultured density must not exceed 30 sea cucumbers per cubic meter. The quality of the holding environment is maintained through regular water changes and close monitoring of temperature, according to the production plan (see Chapter 7 for more information on broodstock conditioning and spawning).
2. Spawning. Release of spermatozoa and oocytes by ripe *A. japonicus* occurs when seawater temperature reaches 16–20 °C in the northern Yellow Sea of China (Zhou et al., 2001). Maximal seed quality is obtained when spermatozoa and oocytes are released naturally (Figure 2.2). When this is not possible, spawning may be triggered using various techniques, such as desiccation, running water, and temperature shock (see Chapter 7). Spawning males should be removed

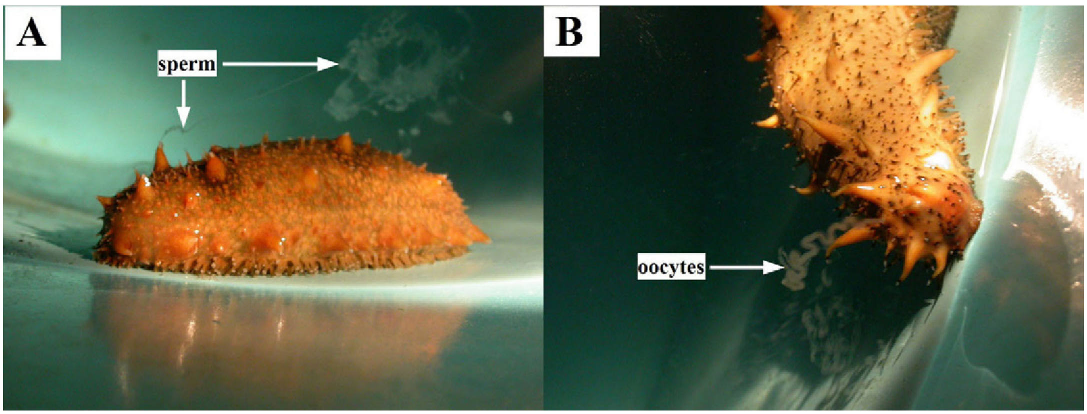

FIGURE 2.2

Spawning behavior of *A. japonicus.* (A) Male releasing sperm. (B) Female releasing oocytes. Animals are about 24 cm long.

Photos by Guangbin Liu

from the pond to avoid excessive accumulation of sperm and polyspermy. For fertilization, spermatozoa density should be 3–5 egg^{-1}, which corresponds to 20–100 mL^{-1}. With this density, the rate of oocyte fertilization in *A. japonicus* is close to 100%.

3. Larval rearing (see Chapters 8 and 16). The fertilized oocytes need to be rinsed with clean seawater. After about 30 h, the fertilized eggs develop into early auricularia larvae. Silk screen mesh is used to transfer the larvae to nursery ponds for cultivation. Larval density should be maintained in the range of $2–6 \times 10^5$ ind. m^{-3}. The auricularia feeds on single-celled algae, such as *Phaeodactylum tricornutum*, *Nitzschia closterium*, *Chaetoceros muelleri*, *Dicrateria inornata*, and *Dunaliella salina*. Microalgae should be mix-fed and can be partially substituted with marine red yeast. The daily feeding dose increases from 1×10^4 cells mL^{-1} in early auricularia stage to $1.5–2.0 \times 10^4$ cells mL^{-1} in late auricularia stage. Regular observation of larvae under a microscope is necessary to monitor development, growth rate, average body length, presence of algae in the stomach, organogenesis, and so on. It is generally advised to add 10 cm of new fresh seawater daily to the culture tank until it is full and then start water changes at a rate of 1/5–1/2 per day, after feeding. Continuous aeration and dark conditions are essential during the rearing period.
4. Larval settlement (see Chapter 8). It takes about 10 days for auricularia larvae of *A. japonicus* to develop into doliolaria larvae. When doliolariae account for about 1/3 of the total population, settlement substrata can be added. The most commonly used substratum is a framework of about $30 \times 50 \times 50$ cm made up of 10–15 layers of corrugated polyethylene sheets, which are spaced out every 6–7 cm. It is worth mentioning that the settlement plates must be conditioned to ensure the growth of benthic diatoms on their surfaces (expected survival rate is between 1 and 30%).
5. Juvenile rearing (Figure 2.3; see Chapter 8). It takes about 20 days from fertilization for *A. japonicus* to settle. Juvenile of *A. japonicus* mainly feed on benthic diatoms present on the settlement plates, supplemented by single-cell algae and other food, such as the filtrate of ground fresh *Sargassum thunbergii*. The feed ration is determined based on juvenile development,

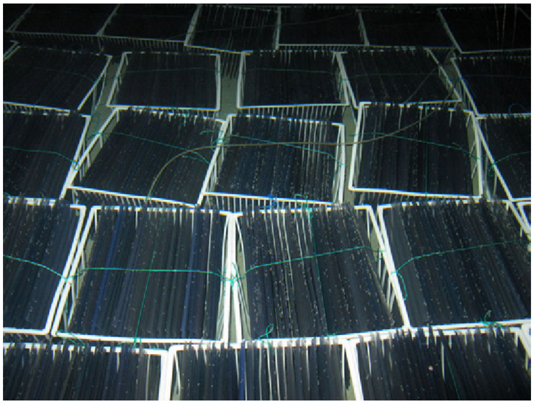

FIGURE 2.3

Juveniles of *A. japonicus* reared in a nursery. The juveniles are feeding on the film of benthic diatoms present on the corrugated settlement plates.

Photo by Shilin Liu

growth rate, and body size. It is recommended to provide one to two complete water changes every day. After about 100 days, juveniles of *A. japonicus* can reach up to 1 cm and can be considered seedlings.

2.2.2 COLLECTION OF WILD SEEDLINGS

In 1954, Zhang Fengying and his team carried out natural seedling collection experiments by placing stones and twig bundles in Beidaihe Bay (at 3–4 m depth) in Hebei Province (China). However, this method only yields limited quantities of wild seedlings, which makes it unsuitable to support the rapidly growing requirements of larger-scale aquaculture. Today, scallop cages are still used to collect seedlings (3–10 cm long) from the wild; however, numbers are still very low with this method.

2.2.3 CURRENT STATE OF *A. japonicus* AQUACULTURE

Research on aquaculture technology used to produce *A. japonicus* started in the 1950s in China. The artificial breeding and rearing of seedlings was done in natural offshore areas in the 1970s. Artificial breeding on larger scales developed in the 1980s. The culture methods and technologies were optimized over the 1990s (Figure 2.4). In the late 1990s, people began to produce *A. japonicus* using large-scale bottom culture (sea ranching) in the coastal areas between Shandong and Liaoning. The scale of aquaculture expanded gradually from 2002, after pond and industrial cultures of *A. japonicus* appeared. In 2003, the total production of *A. japonicus* was about 3×10^4 t, and it increased to 10.22×10^4 t in 2009, over surface areas of 15.53×10^4 ha. The production in Shandong and Liaoning was 6.28×10^4 t and 3.61×10^4 t, respectively, and surface areas cultivated were 4.30×10^4 ha and 10.94×10^4 ha, respectively.

The southernmost region of the current distribution of *A. japonicus* in China is Haizhou Bay, middle of the Yellow Sea; there is no record of natural occurrences of *A. japonicus* in the East China Sea and South China Sea. In the 1950s, *A. japonicus* was transplanted from Dalian, Liaoning Province, to Pingyang County, Wenzhou, Zhejiang Province, and artificial breeding was attempted (Sun et al., 2006). In 2005, seeding and culture were carried out in winter in the coastal region of Fujian Province. In 2008,

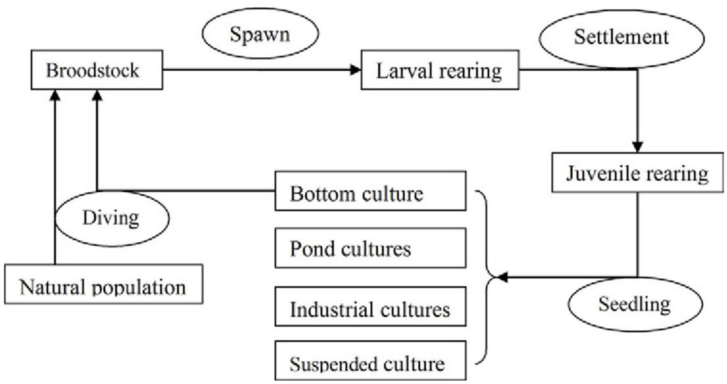

FIGURE 2.4

Typical steps involved in the aquaculture of *A. japonicus.*

Diagram by Guangbin Liu

FIGURE 2.5

Tanks used in the industrial culture of large seedlings (about 25 g) of *A. japonicus* in Yantai, Shandong Province (China).

Photo by Shilin Liu

A. japonicus culture in Fujian expanded rapidly, with economic development and major private capital investment. In 2012, *A. japonicus* culture production in that region was over 2×10^4 t. *A. japonicus* has now been successfully transplanted to Guangdong and Guangxi, the southernmost provinces of China.

Over the past decades, the culture of *A. japonicus* has become one of the most important new aquaculture industries in China's northern coast, valued at over 2×10^{10} Yuan (RMB), equivalent to about 3.27×10^9 US dollars. The culture methods developed from a subtidal bottom culture to different styles of indoor industrial culture (Figure 2.5), pond culture (Figure 2.6), and suspended cultures

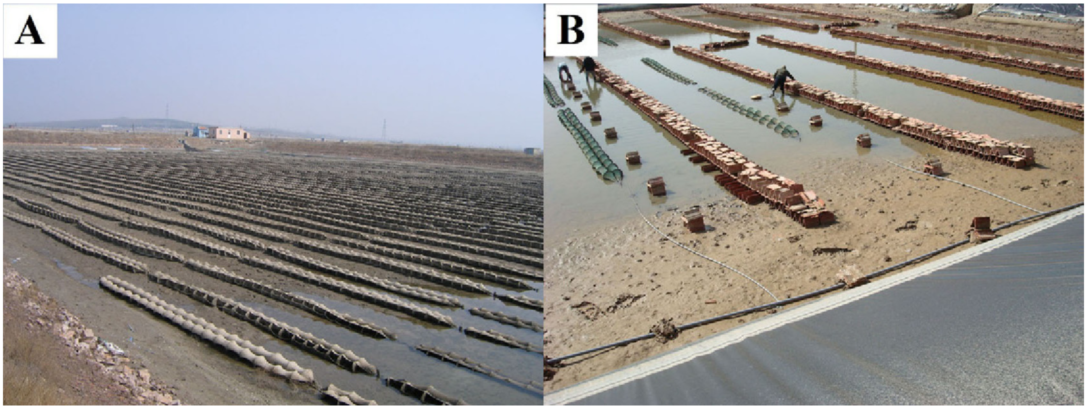

FIGURE 2.6

Aquaculture pond for the growth of *A. japonicus* to commercial size (~200 g) in Qingdao (A) and Dongying (B), both in Shandong Province.

Photos by Guangbin Liu

(see Chapter 16). Meanwhile the culture areas expanded from the northern Yellow Sea to the eastern China Sea.

The rapid development of *A. japonicus* aquaculture requires a great supply of high-quality seed. At present, China and Japan are among the few countries to breed *A. japonicus* in the world (with growing capabilities in Democratic People's Republic of Korea and Republic of Korea) (see Chapters 22–24), and China is the major producer of seed. The main methods revolve around artificial breeding; however, there is still some minor collection of seedlings from the wild.

2.3 PERSPECTIVE ON THE AQUACULTURE INDUSTRY OF *A. japonicus*

2.3.1 STIMULATION OF ECONOMIC AND SOCIAL DEVELOPMENT

The body wall of *A. japonicus* is thick and soft, rich in colloids, polysaccharides, especially acidic polysaccharides with antiaging and antitumor functions (Zhang et al., 2012; Yutaka et al., 2004; Bulgakov et al., 1999), and many trace elements necessary for the human body, such as calcium, magnesium, iron, and manganese and so on (Su et al., 2003; Hu et al., 2000) (see Chapters 13 and 19). Therefore, it is marketed as a health food with beneficial effects on the human body. In recent years, scientists have conducted detailed research into the dietotherapy and pharmacological properties of *A. japonicus*, and its preventive functions are now largely accepted. In China, *A. japonicus* has a long history of consumption, owing mainly to the promotion of its benefits in nourishing *yin*, supplementing kidney, enriching blood, and supplementing insufficiency of the body. Today, Chinese consumers continue to push the demand for *A. japonicus* products despite increasing market costs (see Chapter 21). With the growing wealth in China the consumer market for *A. japonicus* is expected to expand in the years to come.

In 2004, market demand for *A. japonicus* was about 15,000 t, while local production was 5,000 t. Therefore, over half products relied on importation (often other species) from other countries (Chang et al., 2006), including Japan, Russia, Canada, United States, and the island countries of the Indo-Pacific, which make China to be the main importer of sea cucumbers (see Chapters 21 and 22). On the other hand, increasing demands promoted sea cucumbers harvesting in many locations around the world (including *A. japonicus* in China). This led to the decline of *A. japonicus* natural populations in its entire distribution range and to similar situations for other species in other nations (especially in the Indo-Pacific). The price of fresh sea cucumbers increased from 60 Yuan kg^{-1} (~9.8 USD) in 2002 to 150 Yuan kg^{-1} (~24.6 USD) in 2005. There was a drop back to 100 Yuan kg^{-1} (16.4 USD) in 2009 due to the monetary crisis, but prices soared to 230 Yuan kg^{-1} (~37.7 USD) in 2011. The prices of various processed products of *A. japonicus*, including dried body wall (beche-de-mer), dried salted and frozen dry products, and instant sea cucumber remain high, providing favorable market conditions for the culture of *A. japonicus* (see Chapters 20 and 21).

The culture of *A. japonicus* in coastal waters can fetch yields of 15 t ha^{-1} and profits of 6×10^5 Yuan ha^{-1} (~9.8×10^4 USD); profits of 1.4×10^5 Yuan ha^{-1} (~2.3×10^4 USD) in the ponds of northern China; and profits of 400 Yuan m^{-2} (~65.6 USD) in industrial culture. On the coast of Fujian, the profit rates are lower, representing ~30–50% of profits made in northern regions, where the culture originated. The aquaculture industry around *A. japonicus* can already yield higher profits than the aquaculture of other species, including shrimps, crabs, shellfish, and fish in China.

2.3.2 FACTORS LIMITING EXPANSION

The culture of *A. japonicus* is the main pillar of the Chinese mariculture industry, with the largest farming areas and highest output values in the country. However, since 2009, both the farming areas and yields have stagnated for reasons outlined next.

Limited space for development. Sea ranching, pond cultures, and factory farming are the main modes of culturing *A. japonicus*. The need for other economic and social development, including port construction and city expansion, encroaches on the traditional *A. japonicus* culture spaces a little more each year. Along the coastline of Jimo, Qingdao, Shandong Province, about 2000 ha of farming pond surface area have already disappeared to be replaced by urban constructions.

Environmental deterioration (also see Chapter 18). The culture of *A. japonicus* is typically carried out within shallow waters down to 6 m deep in coastal areas susceptible to human impacts, especially those susceptible to red and green tides caused by eutrophication. For example, the so-called green tide of 2008 in Qingdao led to losses of hundreds of millions in *A. japonicus* production and hindered the development of the whole industry.

Diseases (also see Chapter 15). The availability of professional, skilled employees has not kept up with the rapid expansion of *A. japonicus* aquaculture. Therefore, improper management and operational errors may occur that lead to the development of a variety of sea cucumber diseases in aquaculture facilities. Left unmanaged, this situation could lead to heavy economic losses.

Pollution from hatcheries. The underground water resources have been over-exploited to develop cultures of *A. japonicus*. This sometimes led to intrusion of seawater and salinization inland. Pond farming also occupies habitat that may become unsuitable for other local marine organisms. The negative effects of pond farming on the surrounding environment have been getting more attention in recent years.

Aquaculture research lagging behind industrial development. In China, most people culture wild sea cucumbers (rather than selective breeds) that exhibit inconsistent characteristics, such as growth rate, disease resistance, and yield. The epidemic spread of many diseases and pathogens is still not fully understood and the means of detection are not fast or effective enough. Nutrition research is still in its infancy, and artificial diets do not always meet the nutritional requirements of *A. japonicus*. Physiological processes, such as evisceration and regeneration (see Chapter 11), and albinism (see Chapter 12) will require a better understanding in order to develop integrated targeted measures for optimum aquaculture.

Harvesting method for deeper sea ranching. Diving is the traditional method of harvesting sea cucumbers in water 6 m depth or less. However, it becomes increasingly unsafe beyond 10 m, and at depth exceeding 20 m, it can only be accomplished by professional divers. *A. japonicus* can live in coastal waters down to 30 m depth. Relatively deep habitats could therefore be more optimally exploited for sea ranching if a safe and economical harvesting method was developed.

2.3.3 TRENDS IN THE DEVELOPMENT OF *A. japonicus* AQUACULTURE

A. japonicus lives among rocks and macroalgae, where it feeds on tiny organisms, including diatoms, protozoa, small crustaceans, small shellfish, and seaweed fragments (see Chapter 5). Previous studies have shown that when given the choice, *A. japonicus* preferred sediment with high organic content (see Chapters 5 and 10).

Shellfish and macroalgae are the two main species farmed in coastal waters, and their yields accounted for 90% of all the aquaculture products of China. Metabolic products of shellfish are excreted into seawater as pseudofeces that can enrich the sediment and jeopardize water quality. Debris generated by the culture of macroalgae can also generate organic deposits and alter the surrounding environment. Cage culture of fish has similar negative effects on the environment, compounded by the degradation of the artificial feed.

It is therefore expected that *A. japonicus* cultures could be used to reduce microalgal biomass and organic matter content in sediment around other types of cultures (see Chapter 17). Through the experiment of mixed culture including *Chlamys farreri*, *Laminaria japonica* kelp, and *A. japonicus*, it was confirmed that *A. japonicus* could effectively decrease waste load in bottom sediments, and thus improve the economic and ecological conditions, promoting integrated sustainable aquaculture models (Liu et al., 2006; Yang et al., 2000).

The deposit-feeding diet of *A. japonicus* has the potential to play a strategic role in integrated farming systems. It was shown that waste products of 150,000 bay scallops could provide food for *A. japonicus* in the amount of 916.2 kg (Zhao, 2002). At present, development of pond culture and industrial aquaculture is limited. Sea ranching, especially in mixed culture models with shellfish, macroalgae, and/or fish, would not only make full use of limited sea floor space, but also improve aquaculture areas through reduced organic matter accumulation. Therefore, this model offers promising avenues for the development of *A. japonicus* aquaculture in China (see Chapter 17).

Because culture environments may vary considerably from one site to another, rational cultivation modes need to be adopted locally. For example, it was shown that in the Golden Bay of Yantai, an area characterized by frequent storms, rapid currents (flow rates $>0.68\,m\,s^{-1}$), and poor water transparency, developing oyster farming was not ideal, whereas the mixed culture of bay scallop and *A. japonicus* was very suitable (Zhao, 2002). In China, the scale of offshore raft culture is very large: in Shandong Province alone, it represents about 4 million hectares. If this mixed culture was developed in more locations, considerable economic and ecological benefits could be expected.

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CHAPTER

TAXONOMY AND IDENTIFICATION 3

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SUMMARY

Sea cucumbers (Echinodermata: Holothuroidea), also commonly called holothurians or holothuroids, have traditionally been classified through their morphological phenotype, particularly the analysis of their anatomy, including tentacles, papillae, and ossicles (minute calcareous elements). Nowadays, traditional approaches are being complemented by molecular techniques applied to systematics and taxonomy. In the present chapter, we discuss the identification and taxonomy of holothuroids with specific references to *Apostichopus japonicus*. We also describe morphological variability (e.g., color and shape) in *A. japonicus* that complicates the identification of live specimens and dried processed products (beche-de-mer).

Keywords: *Apostichopus japonicus*; classification; evolution; holothurian; holothuroid; sea cucumber; systematics

3.1 INTRODUCTION

The harvest and consumption of sea cucumbers (Echinodermata: Holothuroidea) is long-standing in China, with records that can be traced back some 1000 years. Many ancient Chinese texts on sea cucumber also mention its common use in traditional medicine (see Chapters 1, 19, and 20). The tonic and nutritive virtues ascribed to this sea food has encouraged an ever growing sea cucumber fishing industry, with more than 70 species currently being exploited commercially worldwide, and many sea cucumber populations are now facing drastic declines (see Chapters 5 and 21).

China harbors some 20 commercial species of sea cucumbers. Of these, *Apostichopus japonicus* is the most common and most valuable species. Variations in color and shape, as well as reproductive peculiarities, have been reported over its distribution range, emphasizing the importance of studying population genetics and clarifying regional morphologies. Whether willfully or unintentionally, lower-quality species are sometimes sold as *A. japonicus* on the market; in addition, the sale of fake sea cucumbers is an emerging problem. Once processed (eviscerated and dried), it becomes difficult to identify sea cucumbers through their morphological characteristics. With the development of molecular biology, the detection of fraudulent labeling of sea cucumber products will be made easier.

This chapter reviews advances made in the taxonomic and systematic analysis of sea cucumbers, as well as the various methods used to identify them, with a focus on *A. japonicus* and insights on its particular taxonomic characters.

Zhao, H. 2015. Chapter 3. Taxonomy and identification. In: Yang, H., Hamel, J.-F., Mercier, A. (Eds.), The Sea Cucumber *Apostichopus japonicus*. History, Biology and Aquaculture, Academic Press, pp. 37–52. http://dx.doi.org/10.1016/B978-0-12-799953-1.00003-9

3.2 HOLOTHUROID TAXONOMY

Sea cucumbers were first described by Swedish zoologist Carl Linnaeus in 1758. The type genus of the class Holothuroidea is generally recognized as *Holothuria* Linneaus, 1767. With increasing seafaring and marine surveys, many sea cucumbers were found and classified over the past centuries, and more continue to be discovered to this day (especially in the deep sea). According to Smirnov (2012), holothurians were first considered as the class Scytodermata (Holothrioidea) by Bronn (1860); thereafter, Selenka (1867) first used the name Holothurioidea for a class encompassing these animals and subdivided holothurians into different orders based on the presence or absence of respiratory trees. A year later, Semper (1868) redistributed the species based on the same criteria. Théel (1886) proposed a new system of holothurians on the basis of the presence or absence of tube-feet and their morphology. Ludwig (1889–1892) subdivided the species into different orders based on the embryological origin of the tentacles. While several taxonomic keys were developed over the years, the one defined by Pawson and Fell (1965) is the most commonly accepted and used one. However, the subdivision of the class Holothuroidea into subclasses suggested by Pawson and Fell recently has been demonstrated to be unnatural by Kerr and Kim (2001) and Smirnov (2012).

The classification system of Pawson and Fell (1965):

- Dendrochirotacea
  - Dendrochirotida
    - Placothuriidae
    - Paracucumariidae
    - Psolidae
    - Heterothyonidae
    - Phyllophoridae
    - Sclerodactylidae
    - Cucumariidae
  - Dactylochirotida
    - Ypsilothuriidae
    - Vaneyellidae
    - Rhopalodinidae
- Aspidochirotacea
  - Aspidochirotida
    - Holothuriidae
    - Stichopodidae
    - Synallactidae
  - Elasipodida
    - Deimatidae
    - Laetmogonidae
    - Elipidiidae
    - Psychropotidae
    - Pelagothuriidae
- Apodacea
  - Apodida
    - Synaptidae

Chiridotidae
Myriotrochidae
Molpadida
Molpadiidae
Caudinidae
Eupyrgidae

More than 1400 sea cucumbers constituting 160 genera are found in benthic environments from the intertidal to the deepest oceanic trenches (Smiley, 1994). Due to high floral and faunal diversity in tropical coral reefs, the Indo-West Pacific harbors the richest diversity of holothuroids. Clark and Rowe (1971) recorded 287 species of holothuroids in the shallow waters of the Indo-West Pacific region; at least 35 of them, belonging to the families Holothuriidae and Stichopodidae, are nowadays utilized for production of beche-de-mer in this region. In China, Liao (1997) reported 134 species of sea cucumbers constituting 57 genera. Recently, Liao and Xiao (2011) reported 147 species of sea cucumber in China, also for the most part well distributed in the Indo-West Pacific region.

3.3 METHODS OF CLASSIFYING HOLOTHUROIDS

Holothuroids (or holothurians) can be classified according to their external morphology, such as the tentacles and papillae, along with anatomical characteristics, including the form and arrangements of ossicles (minute calcareous elements in the dermis, sometimes referred to as spicules or sclerites).

The number and shape of tentacles are important factors in holothuroid classification. Dendrochirotida, Aspidochirotida, Apodida, Molpadida, and Elasipodida have 10, 18–30, 12–25, 15, and 10–20 tentacles, respectively. Tentacles of various shapes exist – dendritic, peltate, pinnate, and digitate.

Papillae, the conical prominences on the body wall of some holothuroids, are also used in taxonomy, as the morphology of papillae varies across species. Liao (1997) reported that papillae are obvious in the orders Aspidochirotida and Elasipodida, but inconspicuous in Molpadiida, Dendrochirotida, Apodida, and Dactylochirotida, the other four orders of Holothuroidea. For example, the papillae in *Thelenota ananas*, which are also called caruncles, are large and star shaped. In *Stichopus horrens* the teat-shaped papillae are distributed on the body wall, and in the genera *Peniagone*, anterior dorsal papillae form a raised fan-like prominence (Chang et al., 2011).

The morphology of ossicles is a main focus of holothuroid classification. In order to clearly observe the dermal ossicles, digestion of the body wall is typically needed. The common method involves dissolving the body wall in 5–10% NaOH or 10% NaClO (liquid household bleach) solution until the ossicles are liberated. The ossicles are then rinsed in several changes of distilled water. Another method includes treating the body wall with protease lysis solution ($10\,mmol\,L^{-1}$ Tris-HCl, pH = 8.0; $10\,mmol\,L^{-1}$ EDTA, pH = 8.0; 0.4% SDS; $0.2\,mg\,mL^{-1}$ Protease K) at 50 °C until the ossicles are precipitated. Li et al. (2008) used these three different methods to extract and study the ossicles of 15 commercially important holothuroids. The results showed that ossicles could be obtained by all of these methods with little damage.

Ossicles assume a variety of shapes, for example, rods, perforated plates, buttons, tables, rosettes, cups, anchors, wheels, and C-shaped. Some holothuroids have only one type of ossicle, but others exhibit several types; in this case the proportion of different ossicles is often the basis for classification.

Some researchers have observed and described ossicles in detail, and used them to classify sea cucumbers in China (Wen et al., 2011; Li et al., 2008).

Although traditional taxonomical methods have been widely used, the identification of many holothuroids remains uncertain due to high intraspecific morphological variability and difficulty in extracting taxonomic data from museum specimens. Moreover, with the overfishing of sea cucumbers in recent years, genetic resource protection has received more attention, and an improved phylogenetic classification is becoming essential for a better management of natural populations. DNA nucleotide sequences can now be used to identify sea cucumber species and infer their phylogenetic relationships. Mitochondrial genomic DNA (mtDNA), which evolves at a high rate without recombination, is considered as a potential marker for the identification of species. Inference of phylogenetic relationships of metazoans with complete mitochondrial genome sequences has become increasingly popular, and analyses of entire mtDNA in some species of the phylum Echinodermata have been carried out. The mtDNA sequence length of *Stichopus* sp., *Parastichopus nigripunctatus*, *Holothuria forskali*, and *A. japonicus* is 16257, 16112, 15841, and 16096 bp, respectively (Fan et al., 2012). According to research on mtDNA in sea cucumbers, DNA barcoding is widely used as an effective and useful method for species identification. For example, Arndt et al. (1996) clarified the systematics and taxonomy of some Holothuroidea in the northeast Pacific based on two mitochondrial genes (the large ribosomal subunit and cytochrome oxidase 1). The results showed that *Cucumaria pseudocurata* was distinct from *C. curata* and that *C. fisheriastigmata* should be considered a junior synonym of *C. lubrica*.

Kerr et al. (2005) analyzed eight species from five genera based on conserved 3′ section of 16S mitochondrial ribosomal DNA, and indicated that *Actinopyga* and *Bohadschia* each appear monophyletic, and that *Pearsonothuria* is sister to *Bohadschia*. Byrne et al. (2010) clarified taxonomic uncertainties, species relationships, biogeography, and evolution of the Stichopodidae with phylogenetic analyses of mitochondrial gene (COI, 16S rRNA) sequenced from 111 individuals (7 genera, 17 species). It was found that a monophyly of the genus *Stichopus* was supported with the exception of *Stichopus ellipse*.

3.4 THE EVOLUTION OF HOLOTHUROIDS

Endoskeletal ossicles may be fossilized; they form the basis of echinoderm fossil records and research on the evolutionary history of holothuroids (Croneis and Cormack, 1932; Frizzell and Exline, 1955; Gilliland, 1993). However, limited information has been reported on holothuroid ossicles from China, where only few fossils are found. Zhang (1983, 1985, 1986) reported some holothuroid ossicles obtained from the Lower Carboniferous Shaodong Formation in Hunan Province, and from the Middle Devonian and Early Carboniferous holothuroid ossicles in Hunan Province. Furthermore, Upper Carboniferous holothuroid ossicles from Henan Province were found, as well as Carboniferous-Permian ossicles from Xinjiang Province, Late Triassic ossicles from Western Sichuan and Eastern Xizang provinces, and early Carboniferous and middle-upper Triassic ossicles from Guizhou Province (Zhang, 1993; Hui, 1985; Wang and Chen, 1992; He and Wang, 1995; Chen et al., 2003). Because few holothuroid ossicles are found and those ossicles are sparse, it is hard to compare them with living species. Fell (1965) inferred that the earliest sea cucumber might have evolved from *Helicoplacus* in the Cambrian period or *Eothuria* in the Ordovician period. *Helicoplacus* is spindly, covered with small spiraled

ossicles, and possesses spines (sharp specialized ossicles). *Eothuria* is composed of ossicles similar to those of the Ypsilothuriidae in Dactylochirotida.

Some studies have focused on the evolution of ossicles. Rowe (1969) considered that the ossicles developed from ancestral rosettes and rods to tables and buttons, but Deichmann (1958) and Levin (1999a) inferred that tables and buttons were the ancestral shape of ossicles. Wen et al. (2011) studied the molecular phylogeny and the evolution of ossicles of 15 species within Stichopodidae (including *A. japonicus*) and Holothuroidea based on partial sequences of the 16S rRNA gene. The results showed that tables and buttons were ancestral and later evolved into rods and rosettes. Kerr et al. (2005) studied the molecular phylogeny of eight species within Aspidochirotida, and the results supported increasing ossicle complexity over time.

3.5 *A. japonicus* TAXONOMY

3.5.1 ETYMOLOGY

Selenka (1867) first reported and described this species as *Stichopus japonicus* and *Stichopus armata*, and in 1886 Théel reported this species as *S. japonicus var. typicus*. Augustin (1908) later reported it as *Stichopus roseus*. Mitsukuri (1897) made it clear that these were all synonyms of *S. japonicus*. More recently, Liao (1980) collected this species from Sagami Bay, central Japan, and referred it to a new genus, naming it *A. japonicus*. This species can be found in recent literature under both names (*S. japonicus* and *A. japonicus*). Levin et al. (1985, 1986) and Kalinin et al. (1994) reported major differences between *A. japonicus* and the type species of the genus *Stichopus*, and Levin (1999b) confirmed the need to separate *A. japonicus* from the genus *Stichopus*. This species is now formally accepted within the monotypic genus *Apostichopus* and named *A. japonicus* in the World Register of Marine Species (Paulay, 2013).

3.5.2 MORPHOLOGY AND DESCRIPTION

A. japonicus, the average fresh length of which is approximately 20 cm, has five ambulacra and five interambulacra along the body wall, and four rows of tube feet distributed on the ventral surface, including two rows in the second ambulacrum, one row in the fourth ambulacrum and one row in the tenth ambulacrum. Papillae are distributed in the sixth and eighth dorsal ambulacra and part of the fourth and tenth ventral ambulacra (Figure 3.1). When *A. japonicus* is naturally elongated, a conical prominence appears on the top of the papillae. Different groups of *A. japonicus* have various papillae characteristics. Chang et al. (2011) reported that Chinese populations had four rows of papillae, while Russian and Japanese populations had six rows of papillae (Figure 3.2). To our knowledge, there is no reason to believe that they are different species (shown later). *A. japonicus* possesses 20 tentacles, and its ossicles are dominated by tables. However, the shape and size of ossicles changes with increasing age. The spire of tables in small individuals is higher and the disc larger, with four columns and one to three beams. The tables in adults become vestigial with the loss of spire, and they turn into irregular perforated plates without C-shaped and rosette types (Figure 3.3). The shape of ossicles in this species also varies among different body parts. The cloacal wall has numerous, very

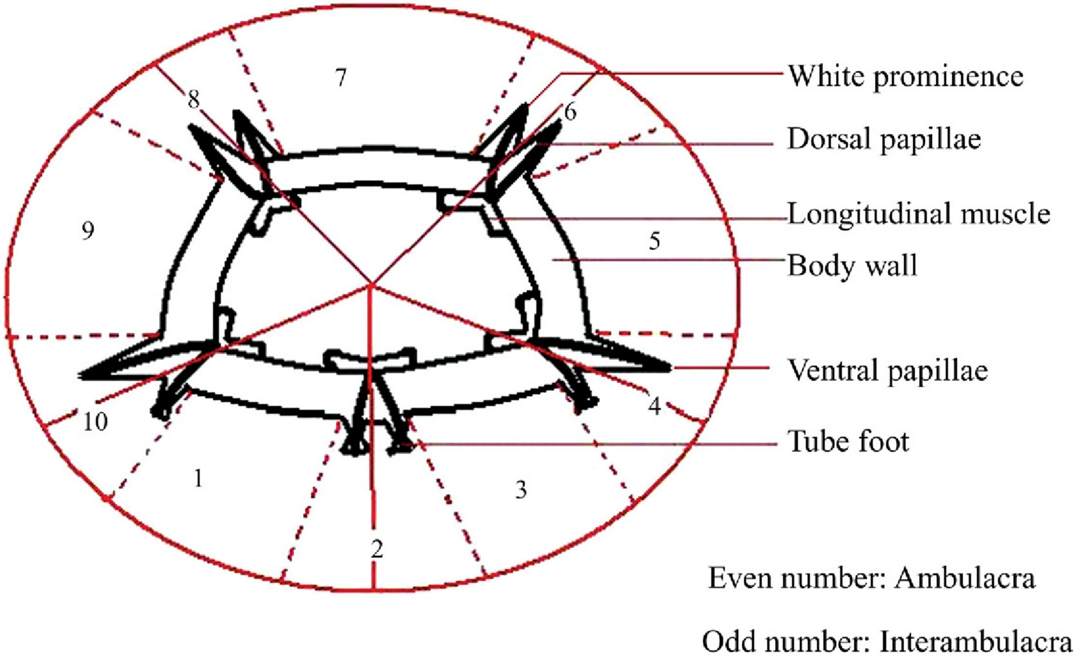

FIGURE 3.1

Diagram of a transverse section of *A. japonicus.*

From Chang et al., 2011

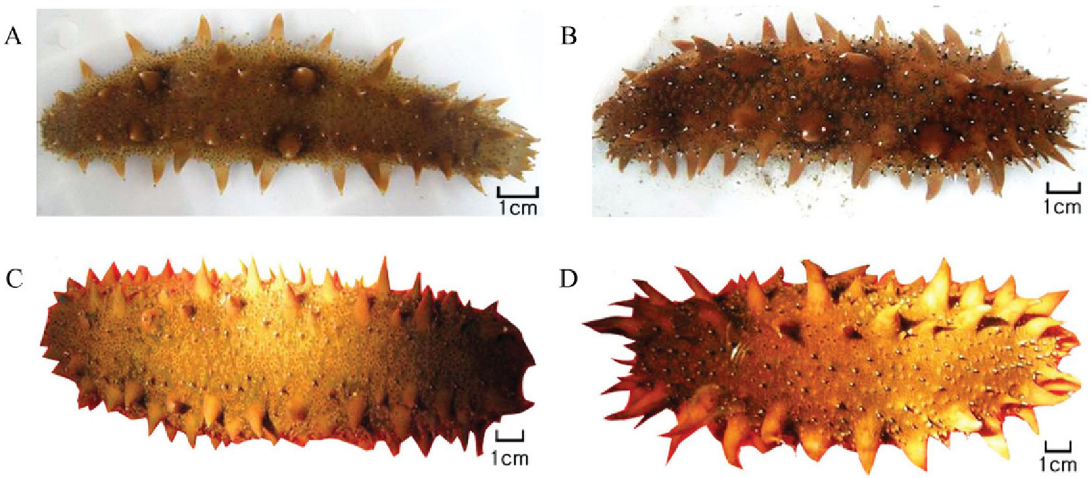

FIGURE 3.2

Appearance of different specimens of *A. japonicus.* (A) Chinese pond-cultured sea cucumber from Zhuanghe, (B) Chinese ♂×Russian ♀ hybrid, (C) Japanese sea cucumber, (D) Russian sea cucumber.

From Chang et al., 2011

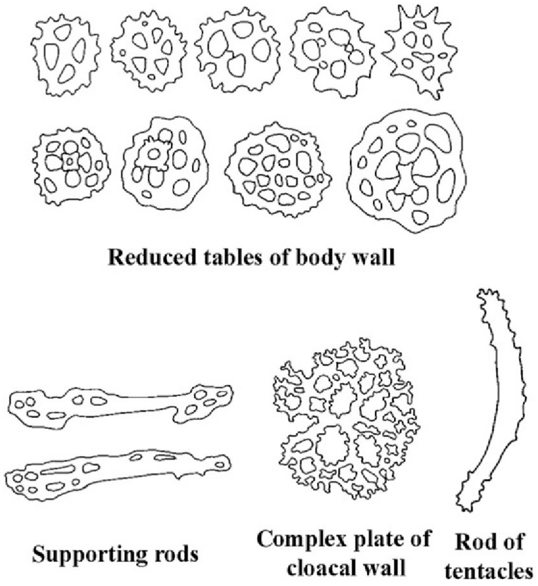

FIGURE 3.3

The various ossicle types found in *A. japonicus.*

From Liao, 1997

complex plates. Ventral podia have similar tables as those found in the body wall and some simple supporting rods. Tables with a more elaborate spire and perforated supporting rods are found in dorsal podia.

The color of the body wall of *A. japonicus* can be defined as red, green, black, or white (Figure 3.4). In China, the body color of native *A. japonicus* is mainly green, while in Japan, red, green, and black variants have been found (Kanno and Kijima, 2002). There are special names to identify these color variants in Japan: *Aka namako* for the red variant, *Namako* for the green variant, and *Kuro namako* for the black (see Chapter 22). The red variants are chiefly associated with offshore gravel beds at depths of 40 m or deeper, whereas the black and green variants are mainly found on muddy and sandy bottoms at shallower depths (Choo, 2008). Some white variants have also been found in China and some researchers have focused on the characteristics of this albino form (Zhao et al., 2012). See Chapter 12 for details on albinism in *A. japonicus*. Using isozyme analysis, Kanno and Kijima (2003) found that the red type and two other color variants had definite genetic differentiation, and Kanno et al. (2006) used microsatellite markers to investigate the genetic relationship among red, green, and black variants, inferring reproductive isolation among them. However, Hu and Li (2009) reported successful hybridization between the green variants from China and the red variants from Japan, suggesting that there was no reproductive isolation between them.

With the development of molecular biology, genomics, and transcriptomics mitochondrial genomes serving as model in genome evolution were used to investigate the systematics of *A. japonicus*. Shen et al. (2009) sequenced the complete mitochondrial genome from *A. japonicus* and examined the phylogenetic relationship among echinoderm classes (Figures 3.5 and 3.6). Sun et al. (2010) sequenced

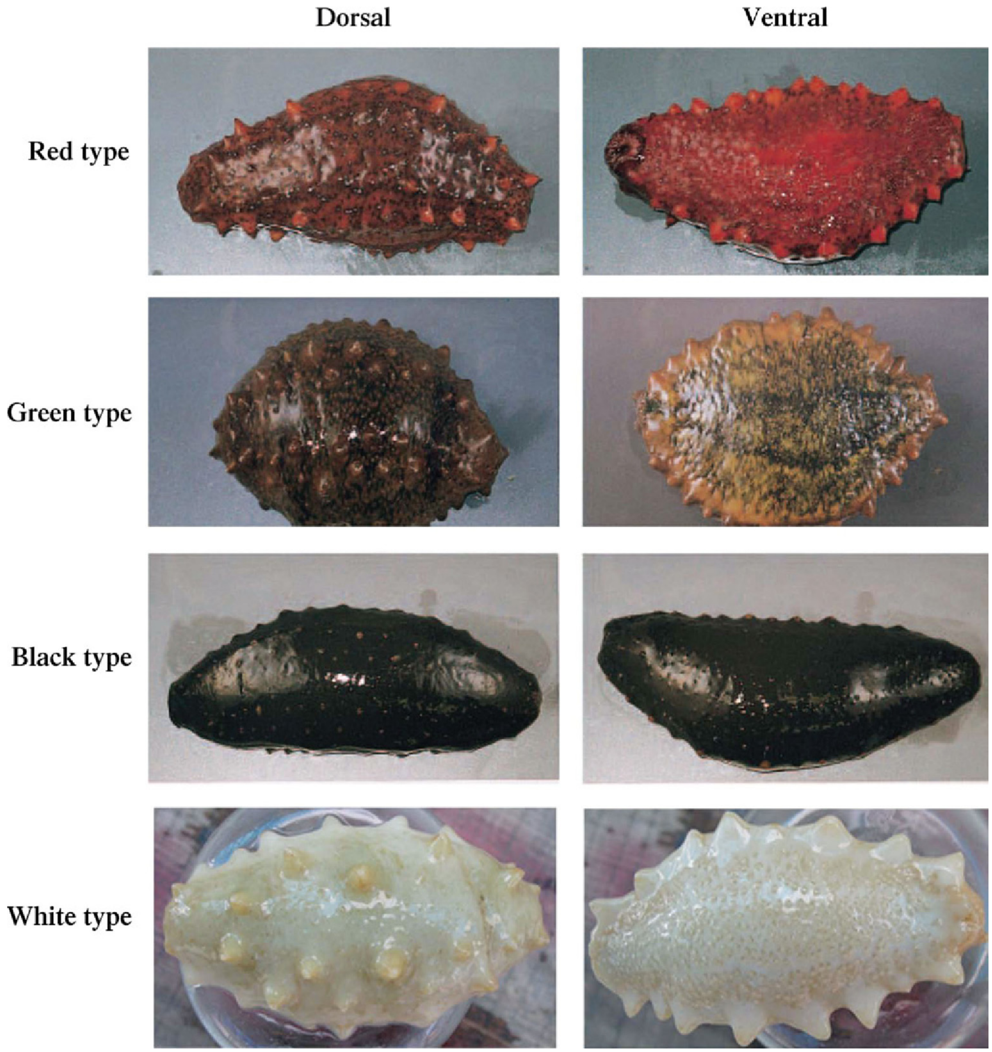

FIGURE 3.4

The different color variants of *A. japonicus.* The left column shows the dorsal surface and the right column the ventral surface of the different sea cucumbers. The length of the sea cucumbers is ~12–13 cm.

The photos of the red, green, and black variants are from Kanno and Kijima (2003); the photos of the white variant are from Liu Shilin

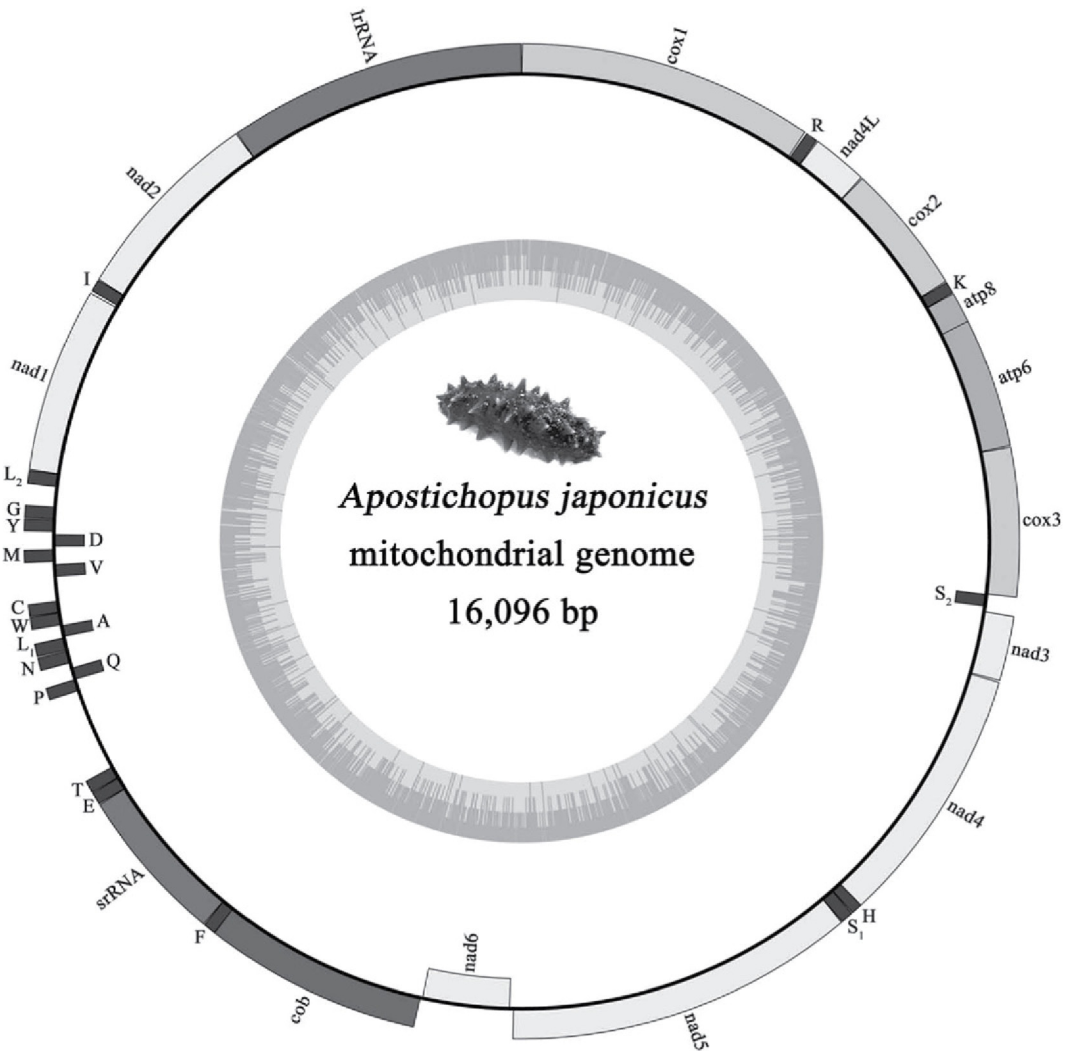

FIGURE 3.5

Gene map of mitochondrial genome of the sea cucumber *A. japonicus.*

From Shen et al., 2009

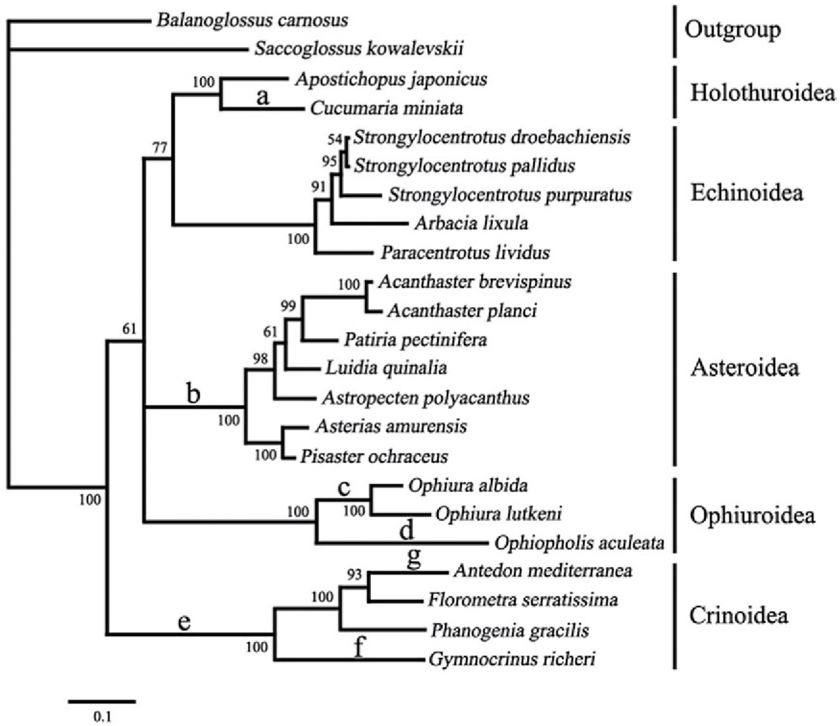

FIGURE 3.6

Phylogenetic tree derived from Bayesian analysis of cob gene (amino acid data) from 21 echinoderms mitochondrial genomes.

From Shen et al., 2009

and compared the red, green, and black color variants of *A. japonicus* mitochondrial (mt) genomes, and found identical gene complements, consisting of 13 protein-coding genes, 22 tRNA genes, and two rRNA genes. The results also showed that the mean genetic distance for the complete mitochondrial DNA of the different color variants is 0.76%, suggesting that the three-color variants indeed belong to the same species. Characterization of the whole transcriptome could provide the most complete gene sets to facilitate future genetic and genomic studies. Sun et al. (2011) recently performed a transcriptome profiling for *A. japonicus* via 454 sequencing and generated more than 23,000 contigs. Du et al. (2012) also performed a large-scale transcriptome profiling for this species, and 29,666 isotigs were obtained and further clustered into 21,071 isogroups.

Kuramochi and Naganuma (2010) reviewed the classification of sea cucumbers belonging to the genus *Apostichopus*, through the authentication of ossicles as well as the observation of external morphology, and some of the specimens that had once been identified as *A. japonicus* were reclassified as *A. armata* (Figures 3.7 and 3.8).

FIGURE 3.7

The comparison of external morphology between *A. japonicus* and *A. armata.* (1a–b) *A. japonicus* and (2a–b) *A. armata*.

From Kuramochi and Naganuma, 2010

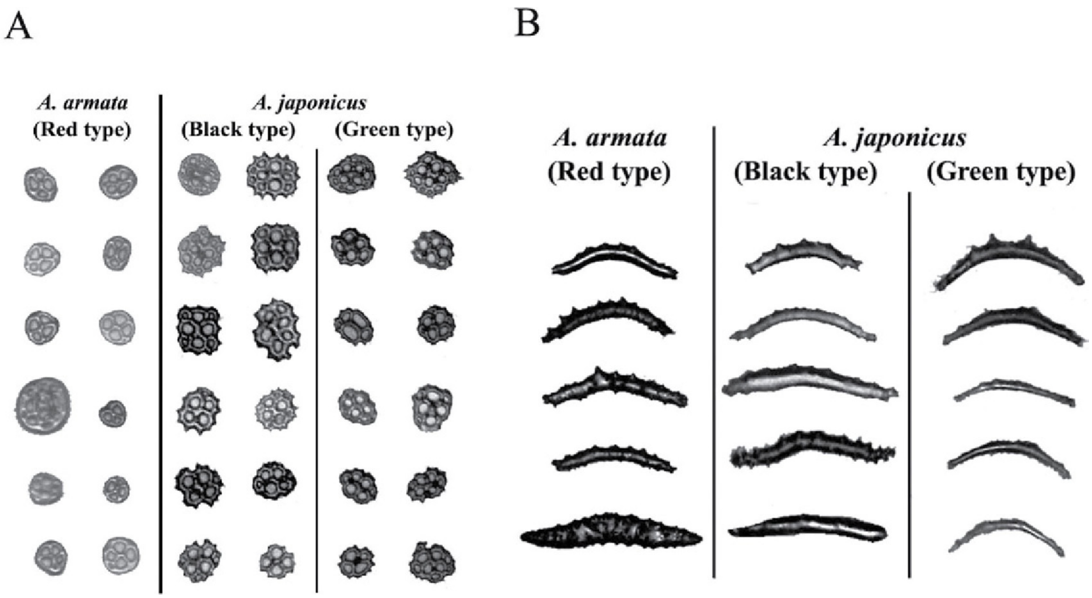

FIGURE 3.8

Comparison of ossicles between *A. japonicus* and *A. armata.* (A) Oar-like ossicles in the body wall. (B) Rod-like ossicles in the tentacles.

From Kuramochi and Naganuma, 2010

Table 3.1 The Different Common Names of *A. japonicus*

Countries	Common Name
English-speaking countries	Japanese sea cucumber, Japanese spiky sea cucumber
France	Bêche-de-mer japonaise
Spain	Cohombro de mar japonés
China	Cishen
Republic of Korea	Dolgi Haesam
Japan	Manamako
Others	Thorn trepang

3.5.3 DISTRIBUTION

A. japonicus, which has different common names (Table 3.1), is mainly distributed in shallow temperate and temperate-cold waters along the coasts of northeastern Asia, including the northern coast of China, Japan, two Koreas, Russia, and Alaska (USA) (Hamel and Mercier, 2008). The northern limits of *A. japonicus*' geographic distribution are the coasts of Sakhalin Island in the Russian Federation and Alaska (Aleutian Islands) in the USA. The southern limit is the island of Tanegashima (Kagoshima Prefecture) in Japan (Figure 3.9). In China, this species is mainly

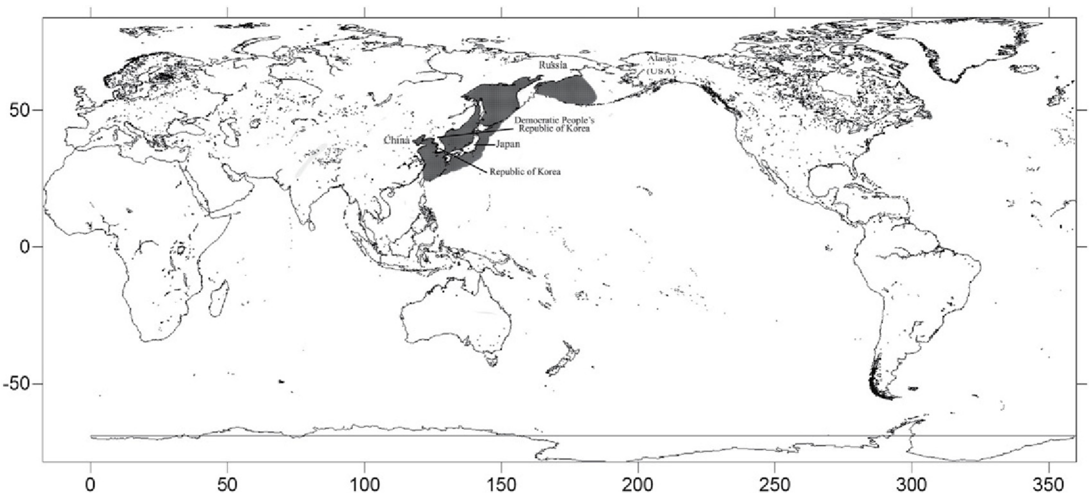

FIGURE 3.9

Geographical distribution of *A. japonicus*.

Modified from Hamel and Mercier, 2008; Purcell et al., 2012

distributed on the coasts of Liaoning, Hebei, and Shandong provinces, and Dalian Island in Lian Yugang; the Jiansu Province marks the southern limit. This species occurs from the shallow intertidal zone to about 20 or 30 m depth (sometimes down to 40 m and a bit more for the red variant). In lagoons of southern Sakhalin Island (Russia), wild populations of *A. japonicus* are found on hard substrates among fields of the red alga *Ahnfeltia tobuchiensis* and in oyster beds (Dubrovskii and Sergeenko, 2002). See Chapter 5 for details on spatial distribution and population structure of *A. japonicus*.

3.5.4 IDENTIFYING PROCESSED PRODUCTS

Sea cucumbers are typically eviscerated, boiled, and dried to be commercialized as beche-de-mer. Cooked and dried sea cucumbers are dark gray to dark brown, and have pointed protrusions in rows along the body (Figure 3.10) (Purcell et al., 2012; Chen, 2005). Once processed, it is sometimes hard to identify sea cucumber species through their morphological characters. Driven by potentially high commercial benefits and the increasing demand for *A. japonicus*, substitution with similar-looking but lower-grade species regularly occurs. Wen et al. (2012) used PCR to identify frozen or dried sea cucumbers, and found that certain products marketed as *A. japonicus* were in fact *Isostichopus* sp. In this context, PCR-RFLP and FINS methods are also useful to detect the fraudulent labeling of sea cucumber products (Wen et al., 2010).

Another type of fraud has recently emerged from poorly regulated trade and high prices fetched by *A. japonicus* in the form of "fake" sea cucumber. Genuine dried sea cucumber tends to be small and of unprepossessing appearance, whereas its fake equivalent is larger and typically translucent. Fake sea cucumber only contains about 20% of real sea cucumber and could pose a threat to human health. A discussion of fake sea cucumber can be found here: http://szdaily.sznews.com/html/2011-09/20/content_1754631.htm (published in the Shenzhen Daily, September 20, 2011).

FIGURE 3.10

Processed appearance of the sea cucumber *A. japonicus*. On the left are fully dried products; on the right are salted semiprocessed products.

From Chen, 2005

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ANATOMY

4

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SUMMARY

Like other sea cucumbers, *Apostichopus japonicus* has a roughly cylindrical shape. It is radially symmetrical along its longitudinal axis, and has weak transversal bilateral symmetry with defined dorsal and ventral surfaces. There are five ambulacral zones along the length of the body from the mouth to the anus – two on the dorsal surface, and three on the ventral surface. The anterior end of the animal, bearing the mouth, corresponds to the oral pole of other echinoderms (e.g., sea urchin and sea star). The posterior end, where the anus is located, corresponds to the aboral pole. *A. japonicus* has a well-developed respiratory tree, but no Cuvierian organ. This chapter describes and discusses the anatomy of the body wall, endoskeleton, coelom and coelomocytes, as well as the nervous, digestive, respiratory, water vascular, hemal, and reproductive systems of *A. japonicus*. The macrostructural, microstructural, and ultrastructural aspects are covered in detail.

Keywords: *Apostichopus japonicus*; body wall; calcareous ring; coelom; coelomocyte; digestive tract; endoskeleton; hemal system; nervous system; reproductive system; respiratory tree; sea cucumber; water vascular system

4.1 BODY WALL

The body of the sea cucumber is surrounded by a relatively thick and often leather-like body wall, which is the main edible organ (processed as beche-de-mer). Five ambulacral areas run along the length of the body from the mouth to the anus. The three on the ventral surface have numerous tube feet, with adhesive discs (Figure 4.1A; Liao, 1997). Like a number of other species, *A. japonicus* also exhibits nodular or verrucous papillae (modified tube feet) on its dorsal surface (Liao, 1997). There are four to six lines of papillae in *A. japonicus* (Figure 4.1B).

The body wall of *A. japonicus* consists of successive tissue layers: cuticle, epidermis, dermis, muscle layer, and coelomic epithelium (Figure 4.2; Liao, 1997). The cuticle is the outer surface of the body wall. It is a thin and structureless layer (Inoue et al., 1999; Liao, 1988). The epithelial cells are long and have a thin base (Figure 4.2; Liao, 1997). There is no clear boundary between the epidermis and the underlying dermis. The dermal layer is the main constituent that determines the thickness of the body wall (Figure 4.2; Liao, 1997). The outer layer of the dermis is composed of loose connective tissue; it forms meshes surrounding the ossicles (microscopic calcareous elements; see Section 4.3). The connective tissue fibers in the inner layer are much denser than in the outer layer

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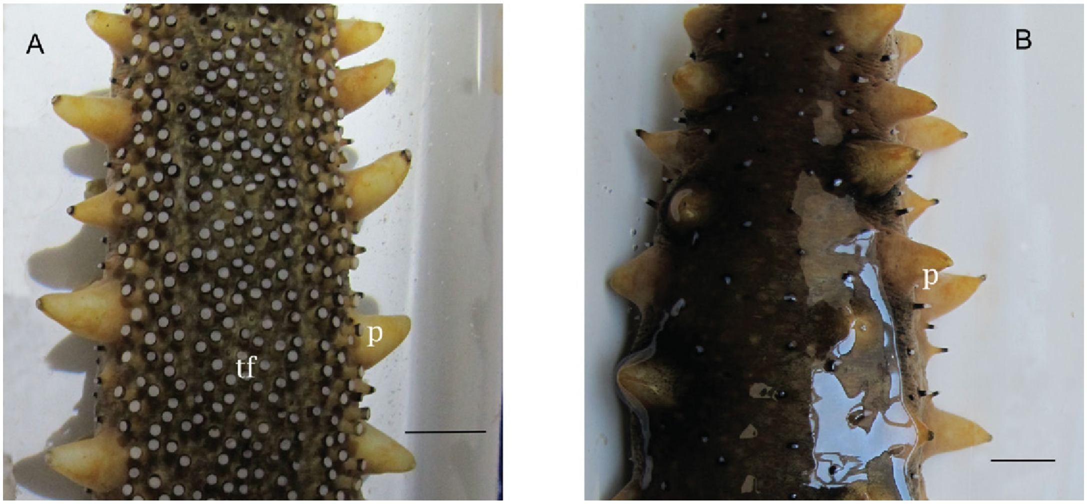

FIGURE 4.1

Body wall, ventral side (A) and dorsal side (B) in a live *A. japonicus*, showing tube feet (tf) and papillae (p). Scale bars = 1 cm.

Photo by Fei Gao

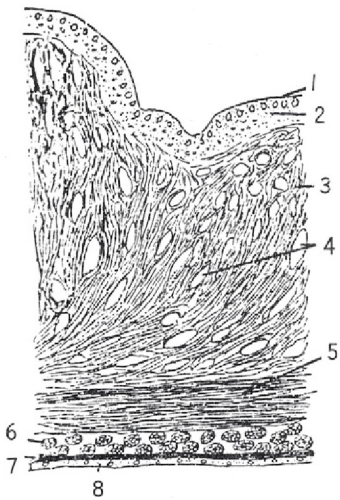

FIGURE 4.2

Drawing of a cross-section of body wall tissue of *A. japonicus*, showing cuticle (1), epidermis (2), loose connective tissue of dermis (3), meshes surrounding the ossicles (4), dense connective tissue in the dermis (5), lacuna with coelomocytes (6), muscular layer (7), coelomic epithelium (8).

From Liao, 1997

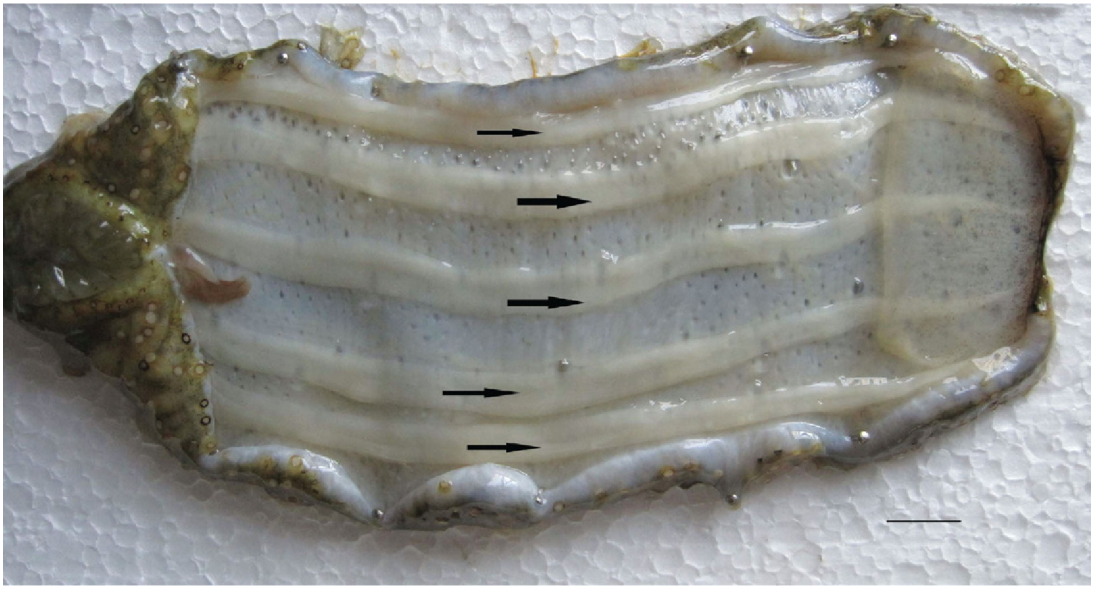

FIGURE 4.3

The five longitudinal muscle bands (arrows) of *A. japonicus.* Scale bar = 1 cm.

Photo by Fei Gao

(Figure 4.2; Liao, 1997). The muscle layer is composed of circular and longitudinal muscles. The circular muscles are the continuous muscle layer below the cortex (Figure 4.2; Sui, 1988; Liao, 1997). The contraction of the circular muscles makes the body wall circumference smaller (Sui, 1988). A bundle of longitudinal muscles is found in each ambulacral zone (Figure 4.3). The anterior ends of the five bundles of longitudinal muscles are attached to the five radial plates of the calcareous ring. The rear ends surround the anus. There is a longitudinal groove in each longitudinal muscle. The contraction of the longitudinal muscles makes the body shorter (Sui, 1988). The body cavity lining is a layer of epithelial cells.

4.2 CALCAREOUS RING

A white circular calcareous ring surrounds the pharynx of *A. japonicus* (Figure 4.4). It is composed of five radial and five interradial plates, the radial plate being larger than the interradial one (Figure 4.5; Sui, 1988; Liao, 1997). The calcareous ring is not completely symmetrical; the height of the plates on the dorsal side is about 1.2 times that of the ventral side. There is a cavity in the anterior end of the radial plate, which is a passage for the radial water canal and the radial nerve (Liao, 1997). The calcareous ring serves as an attachment point for muscles operating the oral tentacles and for the anterior ends of other muscles that contract the body longitudinally.

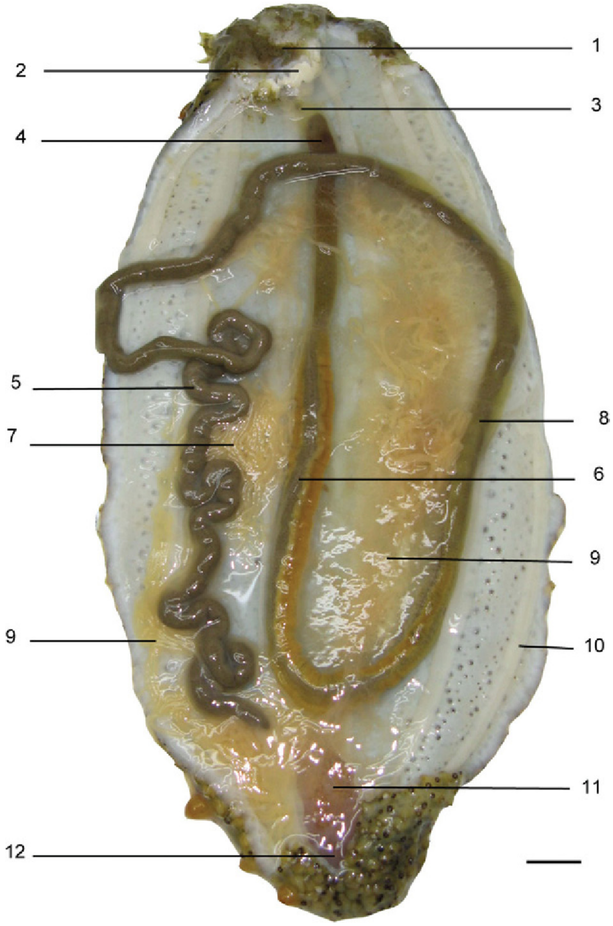

FIGURE 4.4

Internal anatomy of *A. japonicus*, showing mouth (1), calcareous ring (2), esophagus (3), stomach (4), posterior intestine (5), anterior intestine (6), gonad (7), middle intestine (8), respiratory tree (9), longitudinal muscle (10), cloaca (11), and anus (12). Scale bar = 1 cm.

Photo by Fei Gao

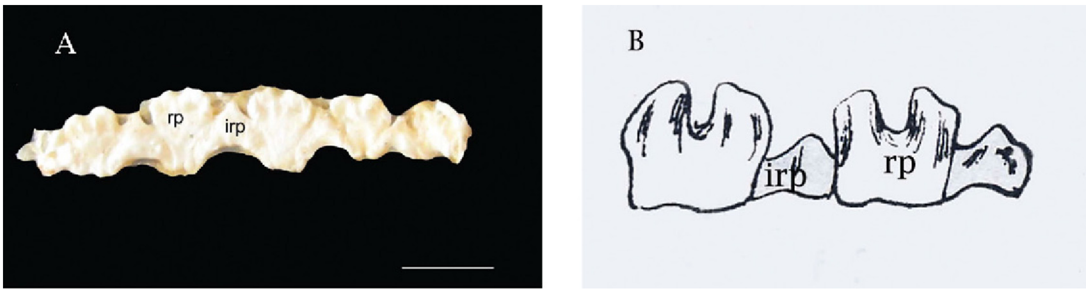

FIGURE 4.5

Photo (A) and diagram (B) showing the calcareous ring of *A. japonicus*, including the radial plate (rp) and the interradial plate (irp). Scale bar = 5 mm.

Photo by Fei Gao and diagram from Liao, 1997

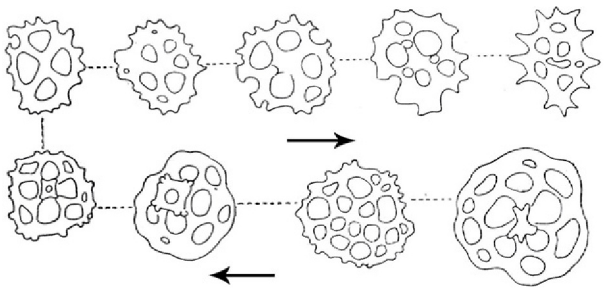

FIGURE 4.6

Morphological changes in the ossicles of *A. japonicus* with age.

From Liao, 1997

4.3 ENDOSKELETON

Calcareous elements, called ossicles, are buried in the connective tissue of the body wall, forming the endoskeleton. Ossicles are a key feature in the taxonomic classification of holothuroids (Sui, 1988) (see Chapter 3 for details). Aside from the larger calcareous ring (see Section 4.2), the ossicles in *A. japonicus* include table-shaped ossicles, rod-shaped ossicles in tube feet and tentacles, complex ossicles around the anus, and large ossicles in the tube feet (Zhu, 2009). The shape of the table-shaped ossicles changes with the age of the sea cucumber (Liao, 1997; Figure 4.6). The lower part of table-shaped ossicles in the juvenile of *A. japonicus* is disc shaped, with a radially porous structure, and the tower part of the table-shaped ossicles is high, constituted by four uprights and one to three beams (Figure 4.7A). In adult *A. japonicus*, the spire part of the table-shaped ossicle disappears (Figure 4.6). In addition, there are spindle-shaped perforated plates in adult sea cucumber (Figure 4.7B; Zhu, 2009). There are rod-shaped ossicles (Figure 4.7C) at the base of the tube feet and papillae. Large perforated plate ossicles are also found in the tube feet (Figure 4.7D). There is another kind of rod-shaped ossicle at the base of the tentacles (Figure 4.7E). Some complex ossicles are located in the dermic layer of the body wall tissues around the anus (Figure 4.7F).

4.4 NERVOUS SYSTEM

The nervous system of *A. japonicus*, like that of most echinoderms, is mainly composed of a circumoral nerve ring and its radial branches (Sui, 1988; Inoue et al., 1999; Figure 4.8A). The nerve ring is located within the peristomial membrane, near the base of the tentacles and in front of the calcareous ring. The nerve ring stretches outwardly into nerves that run along the tentacles, and inwardly to the peristomial membrane and pharynx (Sui, 1988). Radial nerves extend from the nerve ring. The radial nerve is a flat ganglion-shaped cord, and is divided into a thicker outer ectoneural section and a thinner inner hyponeural section by a thin partition of connective tissue (Liao, 1997; Inoue et al., 1999; Figure 4.9A).

The nerves of the tube feet with the epineural sinus emerge from the ectoneural section of the radial nerve and run along each tube foot (Inoue et al., 1999; Figure 4.8B). Unlike the radial nerve, the circumoral nerve ring has only one neural section (Inoue et al., 1999; Figure 4.9B). There is no apparent partitioning between the neural section of the circumoral nerve ring and the ectoneural section of the radial nerve (Inoue et al., 1999; Figure 4.9C). Most of the cell bodies in the nerve ring are found in the periphery of the nerve (Inoue et al., 1999; Figure 4.9B).

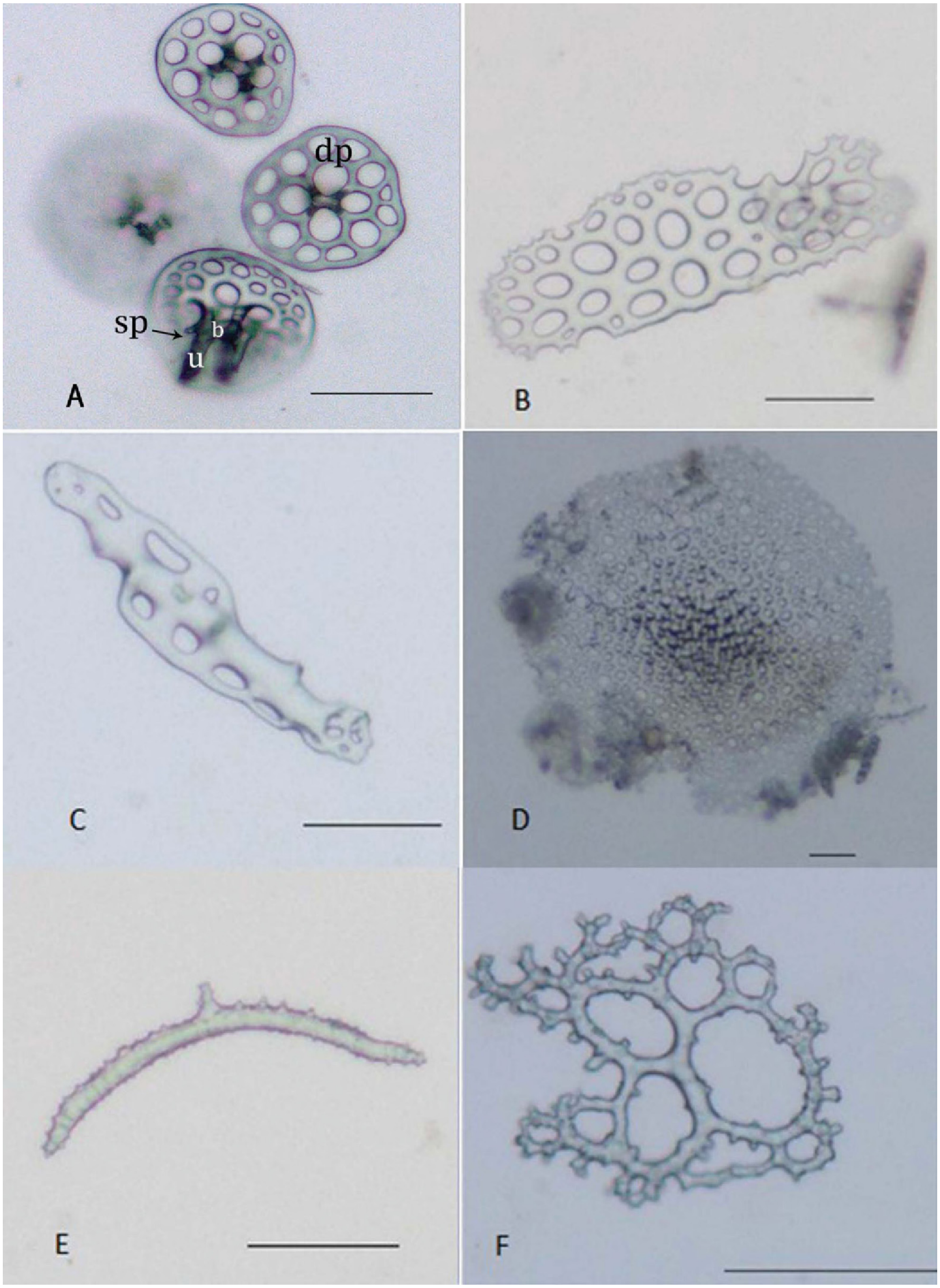

FIGURE 4.7

Ossicles of *A. japonicus.* (A) Table-shaped ossicles; sp, spire part of the table-shaped ossicle; dp, disc part of the table-shaped ossicle; u, upright; b, beam; (B) perforated plate; (C) rod-shaped ossicle in tube foot; (D) large ossicle under the disc of the tube foot; (E) rod-shaped ossicle in tentacle; (F) complex ossicle around the anus. Scale bars = 50 μm.

Photos by Fei Gao

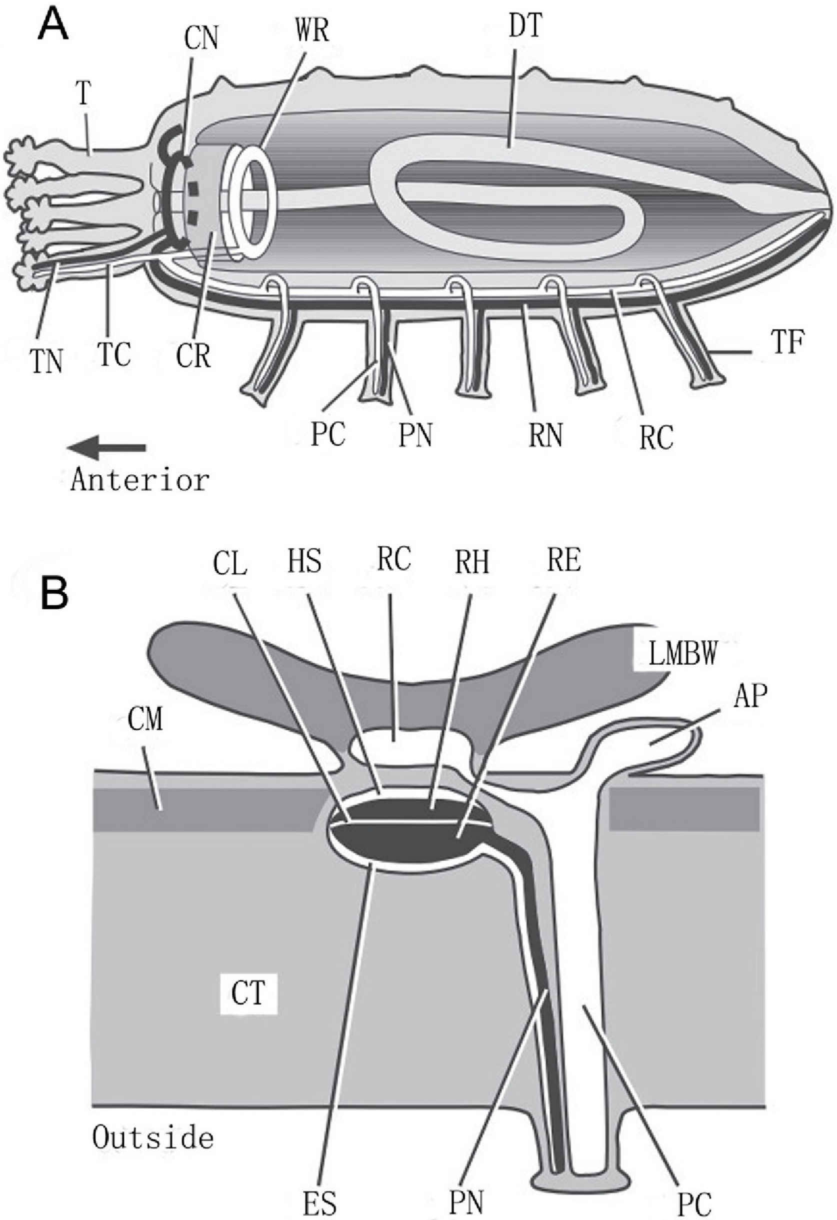

FIGURE 4.8

Diagram showing the nervous system of *A. japonicus.* (A) Lateral view of the whole sea cucumber showing the nervous system, water vascular system and digestive tract. (B) Cross-section of the radial nerve cord. The podial nerve extends from the ectoneural part of the radial nerve cord. AP, ampulla; CL, connective tissue layer; CM, circular muscle; CN, circumoral nerve ring; CR, calcareous ring; CT, connective tissue of the body wall; DT, digestive tract; RE, ectoneural part of the radial nerve; ES, epineural sinus; RH, hyponeural part of the radial nerve; HS, hyponeural sinus; LMBW, longitudinal muscle of the body wall; PC, podial water canal; PN, podial nerve; RC, radial canal; RN, radial nerve; T, tentacle; TC, tentacular water canal; TF, tube foot; TN, tentacular nerve; WR, water ring canal.

From Inoue et al., 1999

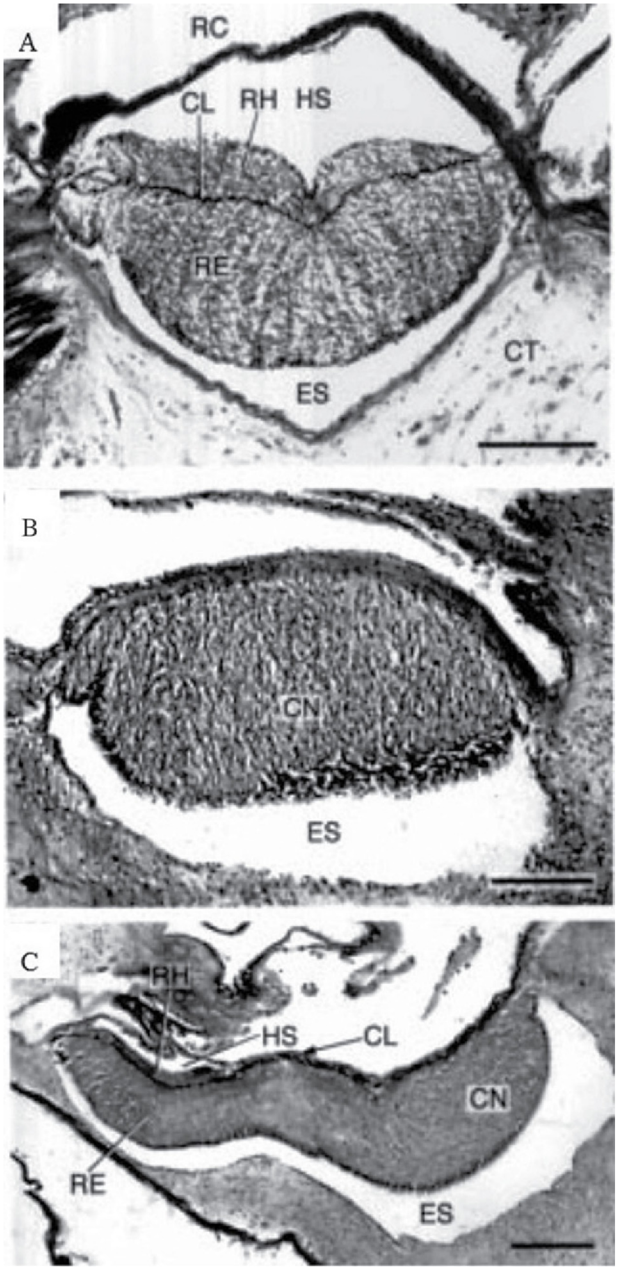

FIGURE 4.9

Cross-section of the radial nerve cord and circumoral nerve ring of *A. japonicus.* (A) Radial nerve cord consisting of the hyponeural and ectoneural nerves separated by a connective tissue layer. Scale bar = 50 μm. (B) Circumoral nerve ring consisting only of the ectoneural nerve. Scale bar = 100 μm. (C) Connection of the radial nerve and circumoral nerve ring. The radial nerve (to the left) consists of distinct hyponeural and ectoneural parts; the latter is continuous with the circumoral nerve ring (to the right); CL, connective tissue layer; CN, circumoral nerve ring; CT, connective tissue of the body wall; ES, epineural sinus; RC, radial canal; RH, hyponeural part of the radial nerve; HS, hyponeural sinus; RE, ectoneural part of the radial nerve. Scale bar = 200 μm.

From Inoue et al., 1999

4.5 COELOM AND COELOMOCYTES

There is a coelomic cavity between the body wall and the visceral mass of *A. japonicus* that holds coelomic fluid, in which many types of coelomocytes are suspended. The known functions of the coelomocytes include mediation of the immune system, trephocytic activities, and nutrient transport (Eliseikina and Magarlamov, 2002).

The types of coelomocytes are characterized based on their morphological and ultrastructural features. Eliseikina and Magarlamov (2002) identified the following types from the coelomic fluid of *A. japonicus*: progenitor cells, amoebocytes, vacuolated cells, small morula cells, morula cells, crystal cells, and vibratile cells. Xing (2009) observed five types of coelomocytes, including round cells, amoebocytes, morula cells, and crystal cells by scanning electron microscopy (Figure 4.10), as well as lymphocytes, amoebocytes, morula cells, and fusiform cells by transmission electron microscopy (Figure 4.11). From a review of the literature combined with the results of their own study, the authors concluded that only lymphocytes, amoebocytes, morula cells, and crystal cells are common cellular elements in the coelomic fluid of *A. japonicus*. Fusiform and vibratile cells are not present in all specimens (Xing, 2009).

4.6 DIGESTIVE SYSTEM

The digestive system of *A. japonicus* is composed of the mouth, pharynx, esophagus, stomach, intestine (anterior intestine, middle intestine, posterior intestine), cloaca, and anus (Figure 4.4; Sui, 1988; Jangoux and Lawrence, 1982).

The length of the digestive tract of *A. japonicus* varies with season. In March, the average digestive tract length of a two-year-old specimen (wet weight, 86–160 g) is 81.5 cm. However, in the aestivation period (in August; see Chapter 11), its average length is only 14 cm (Liu et al., 1996).

4.6.1 MORPHOLOGY

The mouth is located in the center of a peristomial membrane surrounded by a ring of tentacles, which are retractable into the mouth (Figure 4.4). The mouth has sphincters but no trophus.

The pharynx lies behind the mouth, surrounded by the calcareous ring (Figure 4.4). The lower end of the pharynx is the esophagus, which is a yellow, thin, and short section that has no clear boundary with the stomach (Wang, 2004). There are many longitudinal folds in the esophageal wall (Sui, 1988). At the lower end of the esophagus lies the stomach, which is a rust-brown tube (Figure 4.4). The stomach has developed vertical and horizontal folds but has no longitudinal muscle layer (Sui, 1988).

The intestine can be subdivided into anterior, middle, and posterior intestines (Figure 4.4). The anterior intestine is the first descending segment, about 1/4 of the length of the intestine (Figure 4.4; Wang, 2004). There are clearly visible horizontal folds in the inner wall of this section. The middle intestine, connecting with the orange hemal plexus, is an ascending segment, also about 1/4 of the intestine length (Figure 4.4; Wang, 2004). The posterior intestine is the second descending segment, which makes up about 1/2 of the intestine length (Figure 4.4; Wang, 2004). The posterior intestine has a thin wall without horizontal folds. The end of the posterior intestine leads to the cloaca.

The cloaca is a short terminal section of the posterior intestine where the respiratory tree opens. It is short and thick, connecting with the body wall by radial muscles (Figure 4.4; Sui, 1988).

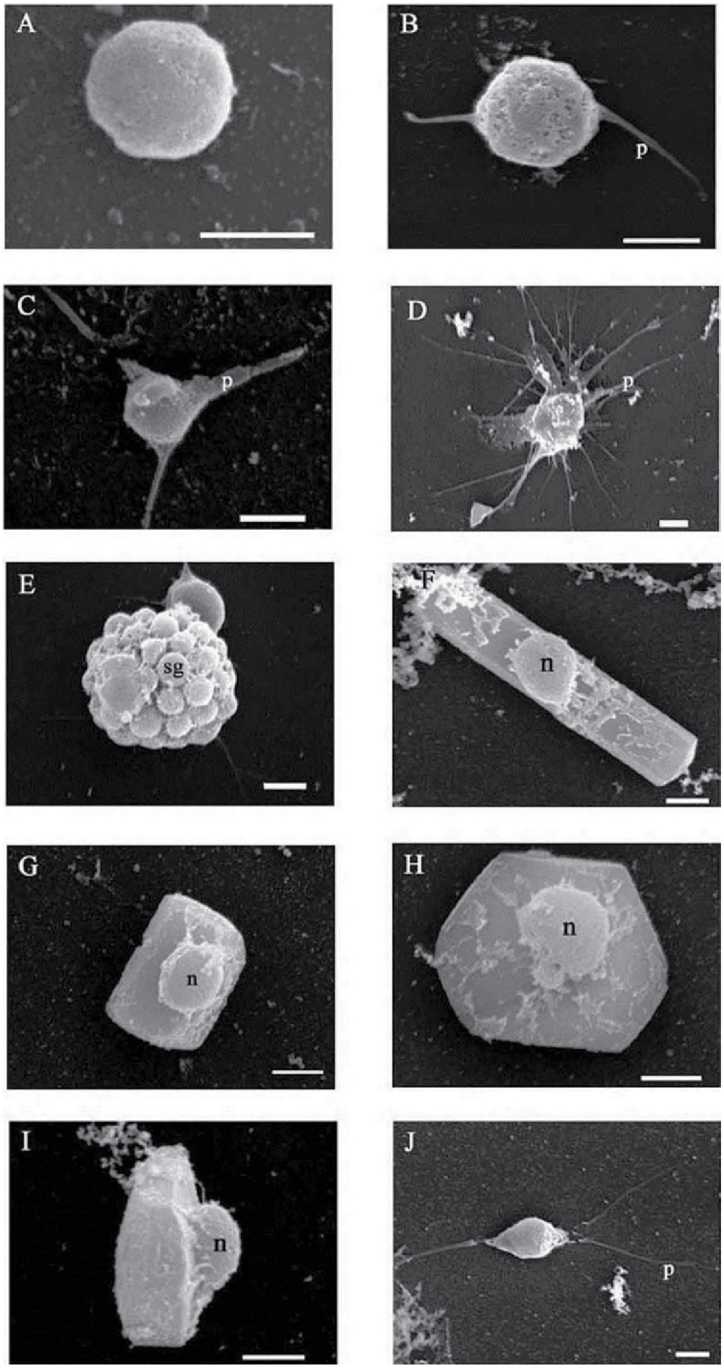

FIGURE 4.10

Scanning electron micrographs of coelomocytes of *A. japonicus.* (A) Round cell; (B) round cell with 2 pseudopodia; (C) round cell with 3 pseudopodia; (D) amoebocyte; (E) morula cell; (F) styloid crystal cell; (G) cuboid crystal cell; (H) hexagon crystal cell; (I) lateral view of a crystal cell; (J) fusiform cell. p, pseudopodia; sg, secretory granule; n, nucleus. Scale bars = 2 μm.

From Xing, 2009

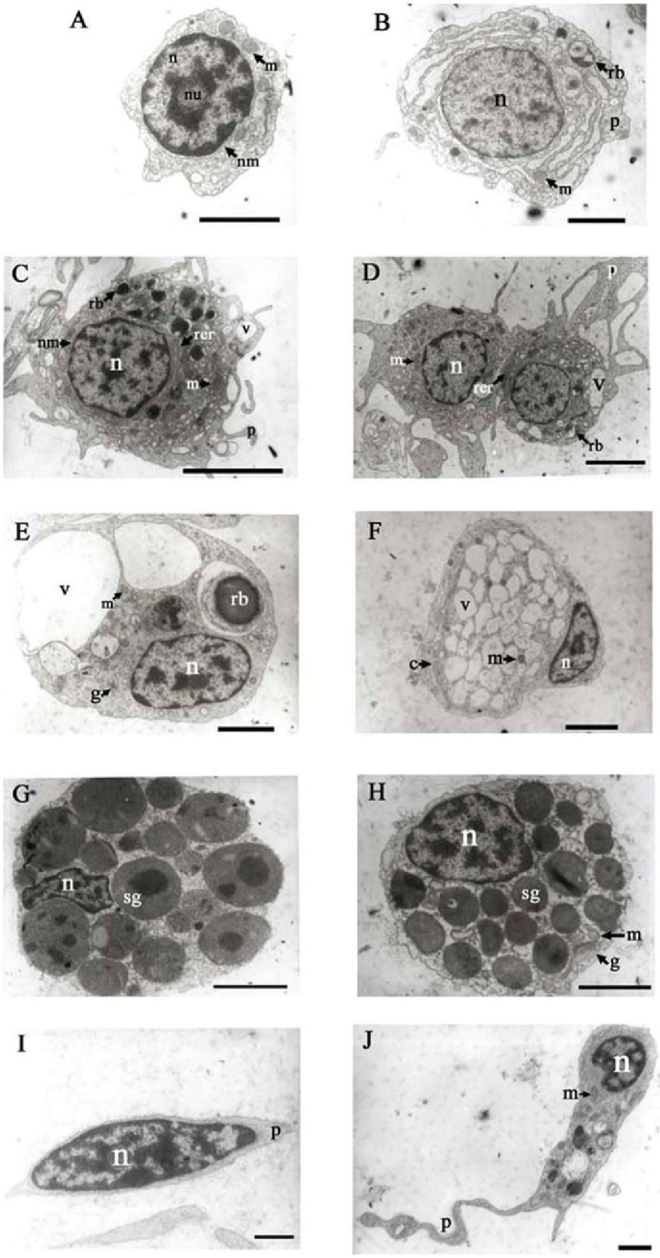

FIGURE 4.11

Transmission electron micrographs of coelomocytes of *A. japonicus.* (A) Lymphocyte with thin cytoplasm; (B) petaloid amoebocyte; (C) filiform amoebocyte; (D) clotting of amoebocytes; (E) an old cell phagocytosed by an amoebocyte; (F) morula cell phagocytosed by an amoebocyte; (G) heterogeneous granules of a morula cell; (H) homogenous granules of a morula cell; (I) fusiform cell; (J) vibratile cell; n, nucleus; nu, nucleoli; nm, nucleus membrane; m, mitochondria; p, pseudopodia; rb, residual bodies; rer, rough endoplasmic reticulum; v, vacuoles; g, Golgi complex; c, cystozooid; sg, secretory granules. Scale bars = 2 μm.

From Xing, 2009

4.6.2 HISTOLOGY

The wall of the digestive tract in *A. japonicus* is comprised of a mucosa, a submucosa, the muscularis, and the tunica adventitia (Cui et al., 2000; Wang et al., 2007). The mucosa is a single-layer or pseudostratified epithelium, composed of columnar, cuboidal, and mucous cells. The submucosa is composed of loose connective tissue. The muscle layer can be divided into the inner longitudinal muscles and outer circular muscles. The tunica adventitia consists of flat cells and a thin layer of connective tissue (Cui et al., 2000; Wang et al., 2007).

4.6.2.1 Esophagus and stomach

The mucosa layers of the esophagus and stomach are made of pseudostratified columnar cells, which form regular longitudinal folds (Cui et al., 2000; Wang et al., 2007). The cells are covered by mucous secretions. The submucosa layer is thin. The outer circular muscles are arranged closely, and are much more developed than the inner longitudinal muscles that are dispersed in the connective tissue of the submucosa (Figure 4.12A; Wang et al., 2007). There are no gland cells in the mucosal epithelium of the esophagus. However, many gland cells are found in the mucosa epithelium of the stomach. The gland cells are large, particularly the nucleus. The cytoplasm is full of secretions that discharge from the top of the cell (Figure 4.12B; Wang et al., 2007).

4.6.2.2 Intestine

The folds of the intestinal mucosa epithelium are composed of simple columnar cells. The top of the cells has a striated border. Some columnar cells secrete substances akin to glycoproteins (Figure 4.12C). The submucosa layer is thinner than the mucosa layer with loose connective tissue. The circular muscles are much more developed than the longitudinal muscles (Wang et al., 2007).

The anterior intestine epithelium has many horizontal folds (Figure 4.12D; Wang et al., 2007) and a small number of longitudinal folds. The middle intestine epithelial folds are irregular. Horizontal folds gradually taper off and many tall and narrow longitudinal folds appear with some branching folds. Only a very small number of columnar epithelial cells secrete substances similar to glycoproteins. The folds in the posterior intestine are comparatively small with only regular longitudinal folds but no horizontal ones. The mucosal epithelium is compose of simple columnar cells and has epithelial cells that do not secrete any glycoprotein-like substances. There are some goblet cells in the posterior intestine mucosal epithelium near the cloaca (Figure 4.12F; Wang et al., 2007).

4.6.2.3 Cloaca

The mucosal epithelium of the cloaca is a simple cuboidal epithelium. There are two kinds of rounded or oval-shaped gland cells present. One kind stains red with hematoxylin–eosin (HE) and has a number of small black particles in it. The other is composed of goblet cells, which appears vacuole-shaped after staining with HE (Figure 4.12G; Wang et al., 2007). The submucosa is thick, while the connective tissue is loose (Wang et al., 2007).

4.7 RESPIRATORY SYSTEM

The respiratory tree of *A. japonicus* is composed of a trunk that branches off the cloaca and extends into the body cavity to the left and right sides of the digestive tract. It is attached to the body wall and intestine by thin strands of connective tissue (Spirina and Dolmatov, 2001). The respiratory tree consists

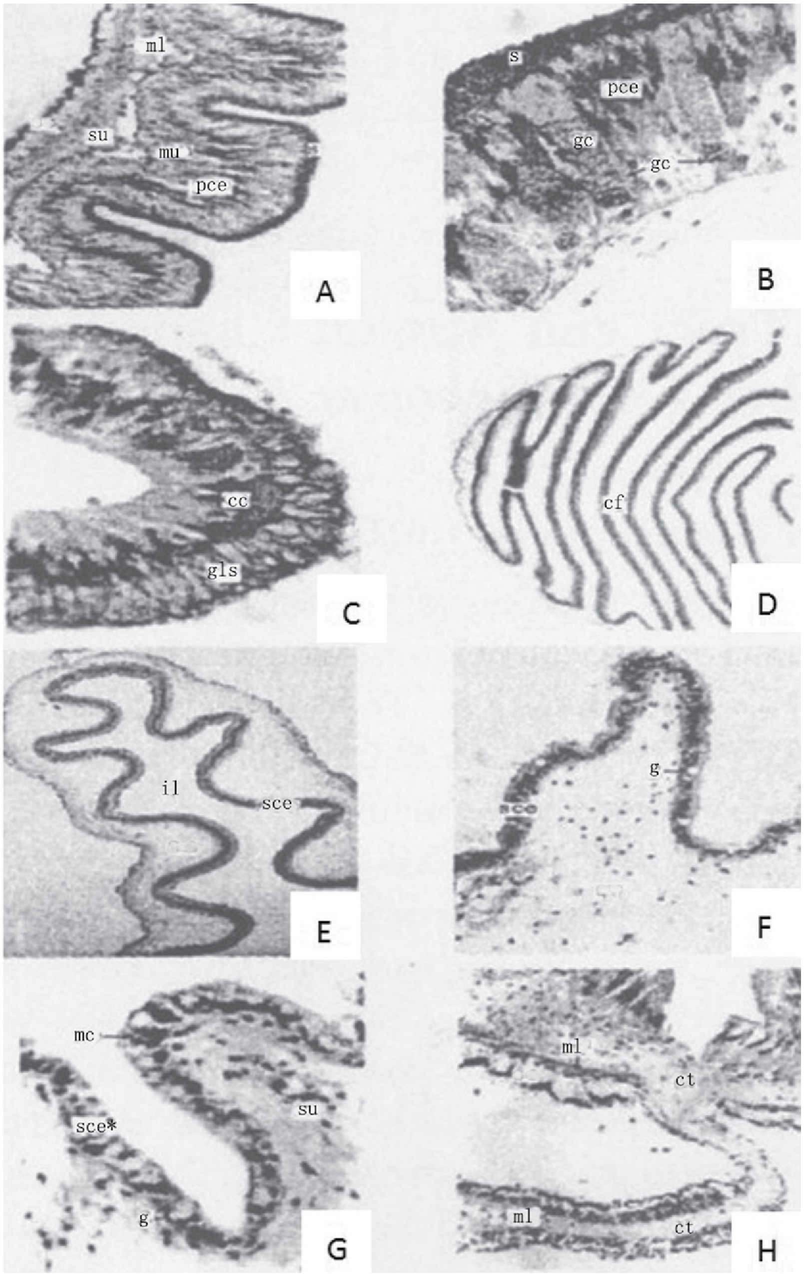

FIGURE 4.12

Histology of epithelium from the digestive tract of *A. japonicus.* (A) Esophagus ×125; (B) stomach ×250; (C) anterior intestine ×250; (D) anterior intestine ×12.5; (E) posterior intestine ×25; (F) posterior intestine ×125; (G) cloaca ×250; (H) esophagus and mesentery ×125. cc, column cell; cf, horizontal folds; ct, connective tissue; g, goblet cell; gc, gland cell in the stomach; gls, secretion-like glycoprotein; il, gut cavity; mc, secretory cell; ml, muscle layer; mu, mucosal epithelium; pce, pseudostratified columnar epithelium; s, secretion; sce, simple columnar epithelium; sce*, simple cuboidal epithelium; su, submucosa.

From Wang et al., 2007

of two dendritic tubules with numerous small caeca branching from a common duct (Sui, 1988). It occupies the bulk of the coelom, extending up to the anterior end of the sea cucumber.

The left dendritic tubule of the respiratory tree is about twofold longer than the right one (Spirina and Dolmatov, 2001). The branches of the left side of the respiratory tree are almost entirely interwoven with the hemal vessels of the rete mirabile (Dolmatov et al., 2011). The right branches do not connect with the hemal sinuses, and are free in the body cavity. The outermost layer of the respiratory tree is covered by a coelomic epithelium with an underlying basal lamina. Under it are a connective tissue layer and the lining epithelium of the respiratory tree cavity with its basal lamina. Hemal lacunae occur in the connective tissue under the coelomic epithelium (Spirina and Dolmatov, 2001).

4.7.1 TRUNK OF THE RESPIRATORY TREE

The lining of the respiratory tree trunk cavity consists of a single-layered folded epithelia (Figures 4.13A, B and 4.14B; Spirina and Dolmatov, 2001). Epithelial cells are attached to the basal lamina by hemidesmosomes. The cells are joined by interdigitations and, in the apical region, by desmosomes (Figure 4.14C; Spirina and Dolmatov, 2001). There are numerous microvilli in the surface of the apical cell, and a single cilium is found in the folds. The nuclei are rounded and located in the central part of the

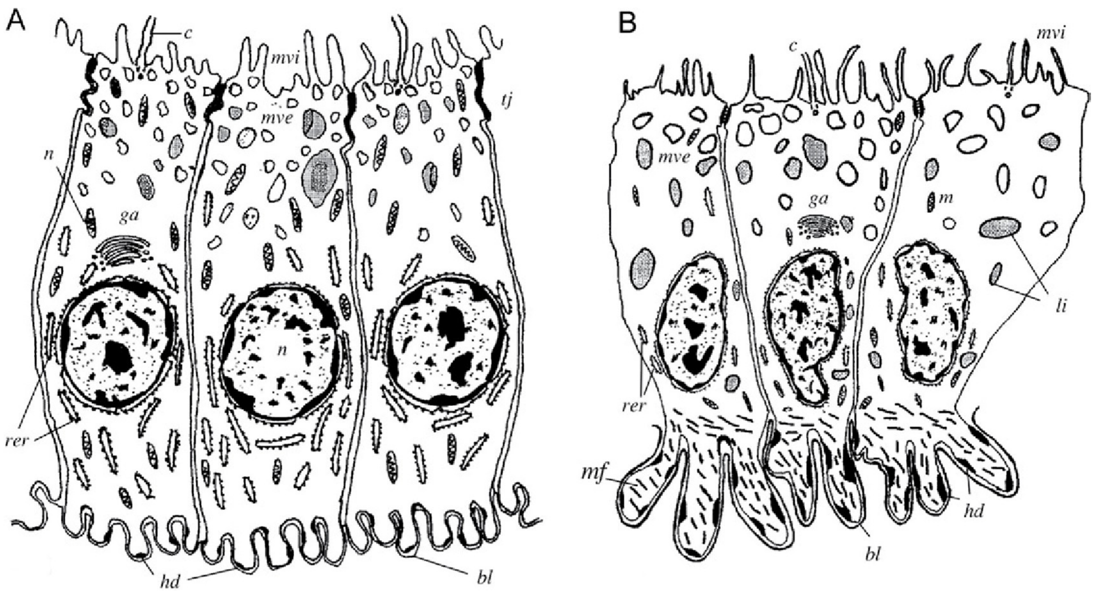

FIGURE 4.13

Schematic representation of the structure of the respiratory tree trunk in *A. japonicus.* (A) Lining epithelium; (B) coelomic epithelium. ga, Golgi apparatus; bl, basal lamina; d, desmosome; li, lipid inclusions; m, mitochondria; mvi, microvilli; mve, microvesicles; mf, myofibrils; hd, hemidesmosomes; tj, tight junctions; c, cilium; rer, rough endoplasmic reticulum; n, nucleus.

From Spirina and Dolmatov, 2001

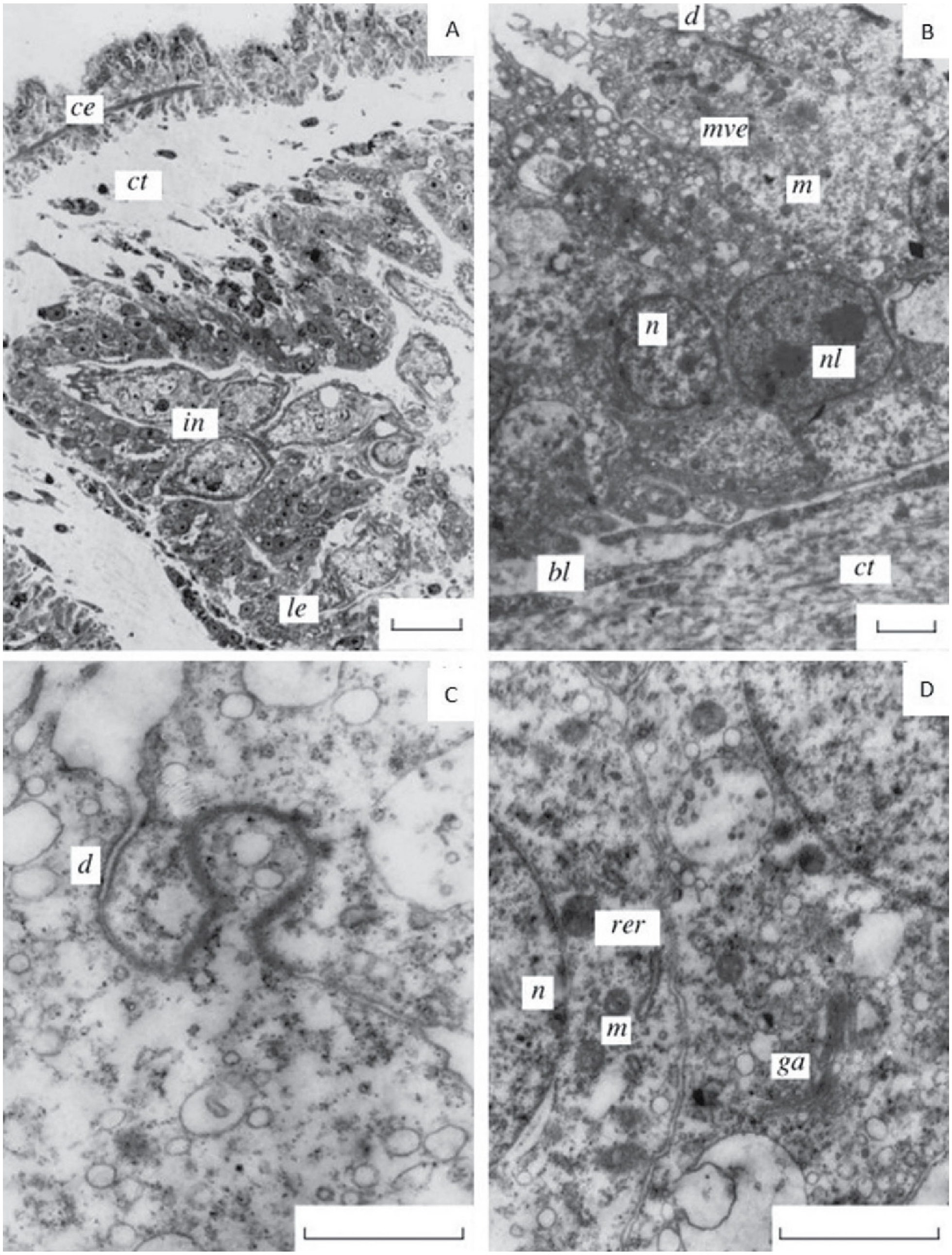

FIGURE 4.14

Epithelium of the cavity of the respiratory tree trunk of *A. japonicus*. (A) General view; (B) lining epithelium; (C) cellular interdigitation; (D) endoplasmic reticulum and Golgi apparatus. ga, Golgi apparatus; bl, basal lamina; le, lining epithelium; d, desmosome; in, infusoria; ct, connective tissue layer; ce, coelomic epithelium; rer, rough endoplasmic reticulum; n, nucleus; nl, nucleolus; mve, microvesicles. Scale bars: A = 50 μm; B = 2 μm; C and D = 1 μm.

From Spirina and Dolmatov, 2001

cells. The apical cytoplasm is full of numerous microvesicles. There are many mitochondria and a well-developed endoplasmic reticulum (Golgi apparatus) in the cytoplasm (Figure 4.14D; Spirina and Dolmatov, 2001).

About half the width of the wall of the respiratory tree is a connective tissue layer made up of amorphous (intercellular) material and thin collagen fibers (Figure 4.15A). The amorphous constituent of the connective tissue is probably composed of glycosaminoglycans (Spirina and Dolmatov, 2001).

The outermost layer of the respiratory tree trunk is lined by a single-layer columnar epithelium, which develops small invaginations into the underlying connective tissue (Spirina and Dolmatov, 2001; Figures 4.13B and 4.15A). Epithelial-muscle cells constituting the bulk of the epithelium rest on the well-developed basal lamina. The cells are attached to the basal lamina by hemidesmosomes and are joined to one another by interdigitations. The basal part of the cells, containing myofibrils, is submerged in the connective tissue and contains myofibrils (Spirina and Dolmatov, 2001; Figure 4.15A). Myofibril bundles can be oriented along the axis of the organ and perpendicularly to it, thus forming the longitudinal and circular musculature of the respiratory tree. There are microvilli and cilia on the surface of the cell. In the cytoplasm, there are moderately developed systems of the rough endoplasmic reticulum and Golgi apparatus and many lipid inclusions (Spirina and Dolmatov, 2001; Figure 4.15B).

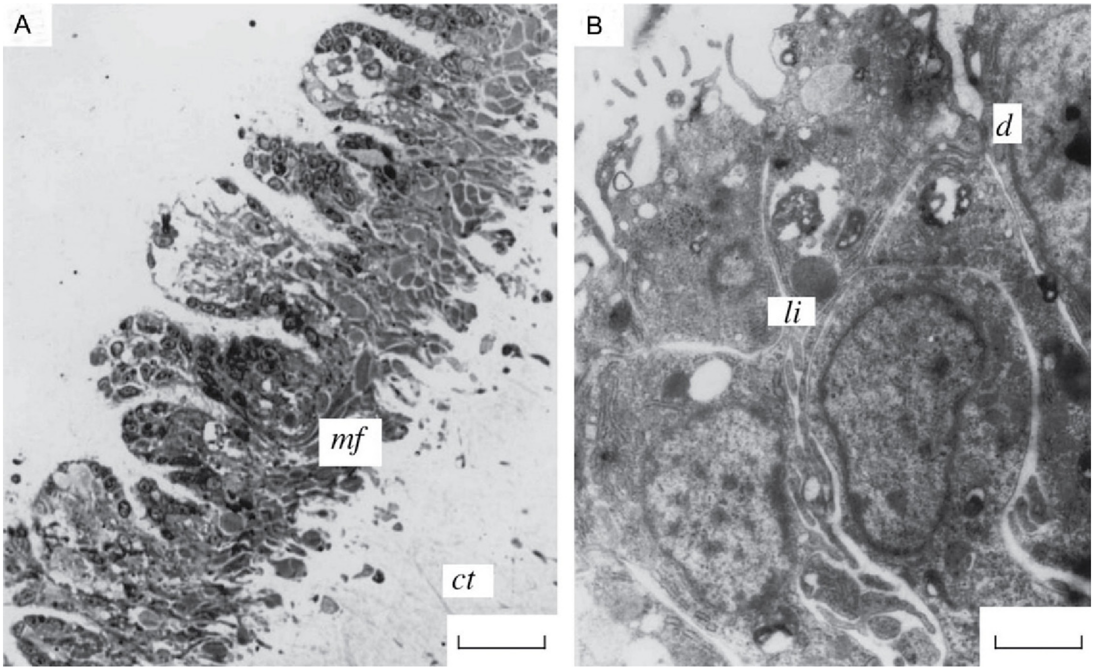

FIGURE 4.15

Coelomic epithelium of the respiratory tree trunk in *A. japonicus*. (A) General view and (B) electronogram; d, desmosome; li, lipid inclusions; mf, myofibrils; ct, connective tissue layer; n, nucleus. Scale bars: A = 50 μm; B = 2 μm.

From Spirina and Dolmatov, 2001

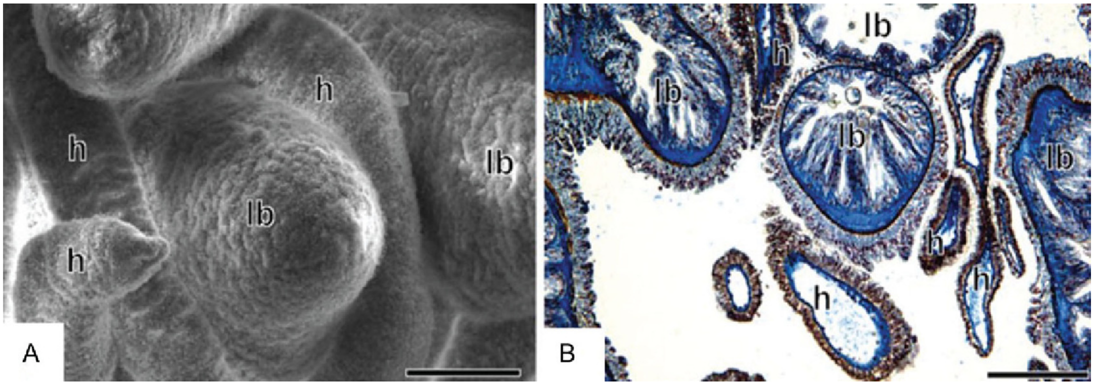

FIGURE 4.16

Interlacing of the respiratory tree with the hemal vessels of the gut in *A. japonicus.* (A) Scanning electron micrograph and (B) light micrograph of interlacing area between hemal vessels (h) of the rete mirabile and lateral branches (lb) of the left respiratory tree. Scale bars = 100 μm.

From Dolmatov et al., 2011

4.7.2 BRANCHES OF THE RESPIRATORY TREE

The structure of the respiratory tree branches is somewhat different from that of the trunk. The epithelia lining the inner cavity of the organ develop fingerlike folds and lean on a fairly thick basal lamina. The connective tissue is composed of a thin layer of intercellular substances in which hemal lacunae occur. The outermost layer of the wall consists of coelomic epithelia (Spirina and Dolmatov, 2001).

4.7.3 CONTACT OF THE RESPIRATORY TREE WITH THE HEMAL VESSELS

The respiratory tree of *A. japonicus* is closely associated with the hemal vessels (Spirina and Dolmatov, 2001; Dolmatov et al., 2011; Figure 4.16A). At the intersection, the epithelium sharply decreases in thickness, and the lining is 1.6–6.25 μm and the coelomic epithelium is 0.42–1.04 μm (Spirina and Dolmatov, 2001). Although the coelomic epithelia of the respiratory tree and hemal vessels are adjacent to each other at the intersection, there is still a 0.1–1.3 μm wide gap (Spirina and Dolmatov, 2001; Dolmatov et al., 2011; Figure 4.16B).

4.8 WATER VASCULAR SYSTEM

Like other echinoderms, *A. japonicus* possesses a water vascular system that provides hydraulic pressure to the tentacles and tube feet, allowing them to move and collect food items. The water vascular system is basically composed of a ring canal and five radial canals (Figures 4.8 and 4.17; Sui, 1988).

The ring canal is colorless and transparent, and is located in the rear of the calcareous ring around the esophagus. A tapering sac, called the Polian vesicle (Figure 4.18A), connects with the ring canal by a narrow neck on the ventral side of the ring canal. There is only one Polian vesicle in *A. japonicus*

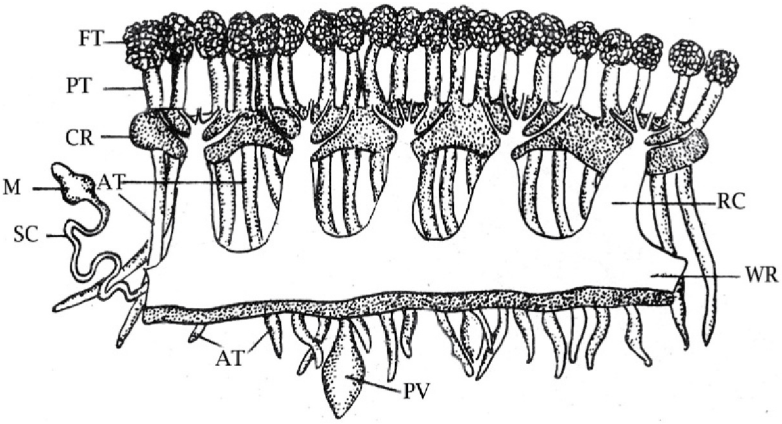

FIGURE 4.17

Diagram of the water vascular system in *A. japonicus*. FT, front end of tentacle; PT petiole of tentacle; AT, ampulla of tentacle; CR, calcareous ring; RC, radial canal; WR, ring canal; PV, Polian vesicle; SC, stone canal; M, madreporite.

From Liao, 1997

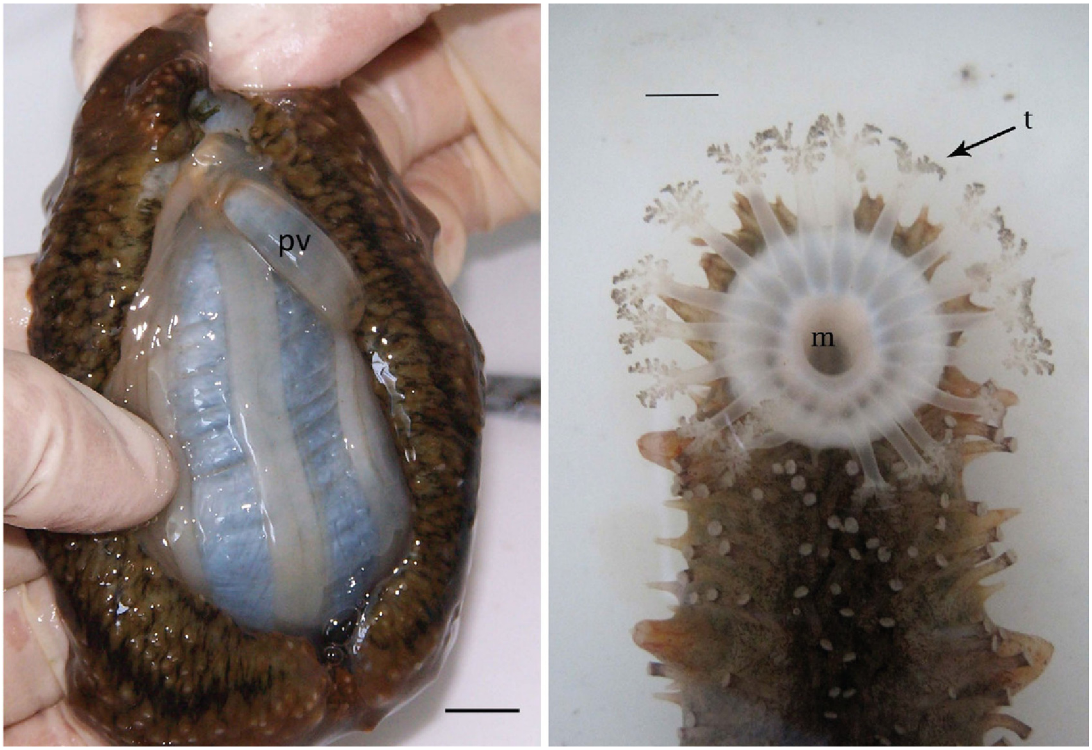

FIGURE 4.18

Polian vesicle and tentacles of *A. japonicus.* (A) Polian vesicle (pv); (B) tentacles (t) around the mouth (m). Scale bars = 1 cm.

Photos by Fei Gao

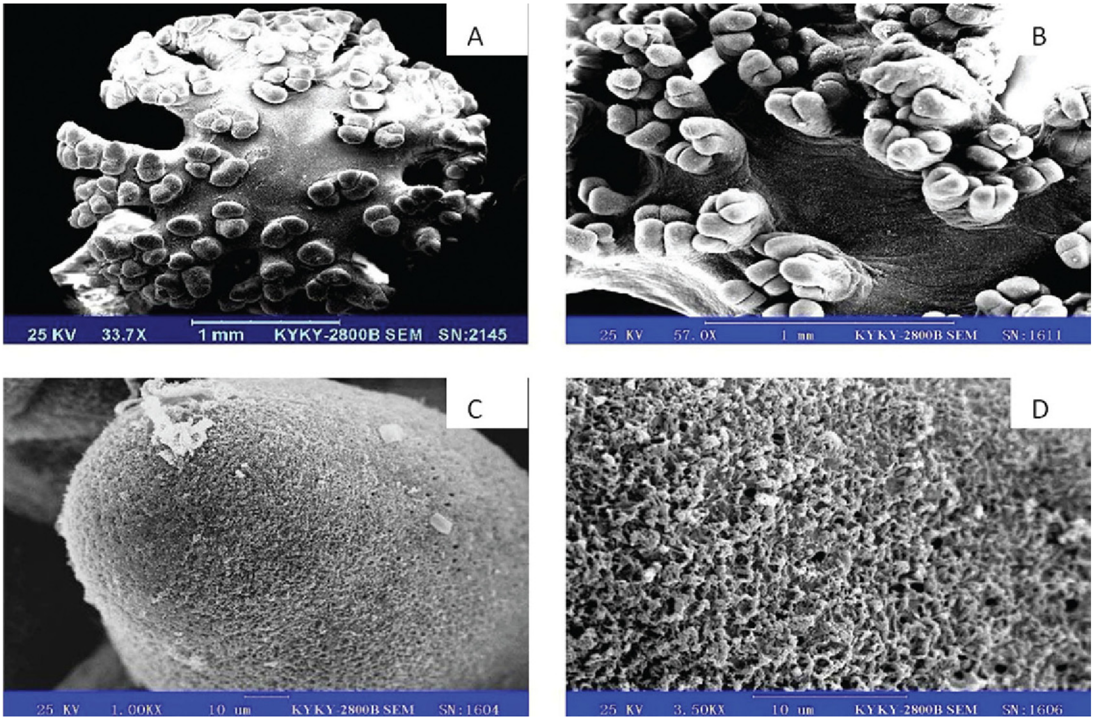

FIGURE 4.19

Surface microstructure of the tentacle of *A. japonicus* observed by scanning electron microscopy. (A) Scutiform tentacles; (B) mastoid processes on the scutiform tentacles; (C) mastoid processes and adhering particles; (D) microvillus on the mastoid processes.

From Zhao, 2010

(Sui, 1988). It is generally recognized that the Polian vesicle can adjust the water pressure in the canal (Sui, 1988). The histological structure of the Polian vesicle is similar to that of the ring canal; however, its wall is much thinner (Liao, 1997).

In the dorsal part of the ring canal, there is a white crooked tubule with a calcified wall, called the stone canal. The madreporite is present at the end of the stone canal, lying within the body cavity, just below the pharynx. The madreporite is usually the passageway through which seawater enters the body. Because the madreporite is located in the coelomic cavity of sea cucumbers, the fluid in the canal is provided by the coelomic fluid (Liao, 1997).

The ring canal divides anteriorly into the tentacle canal. *A. japonicus* possesses 20 oral tentacles (Liao, 1997; Zhao, 2010; Figure 4.18B), subdivided into petiole and scutiform tentacles. There are eight major branches at the end of each tentacle, and each major branch stretches out into secondary branches, tertiary branches, or slender branches (Figure 4.19A). Some mastoid processes are distributed on the branches (Figure 4.19B); there are microvilli and ostioles on their surface (Figure 4.19C, D; Zhao, 2010).

The petiole part of the tentacle consists of cuticle, epithelial layer, loose connective tissue, dense connective tissue, nervous layer, muscular layer, and coelomic epithelium lining (Figure 4.20A; Zhao, 2010). There are one to two layers of dense connective tissue cells, close to the nervous layer,

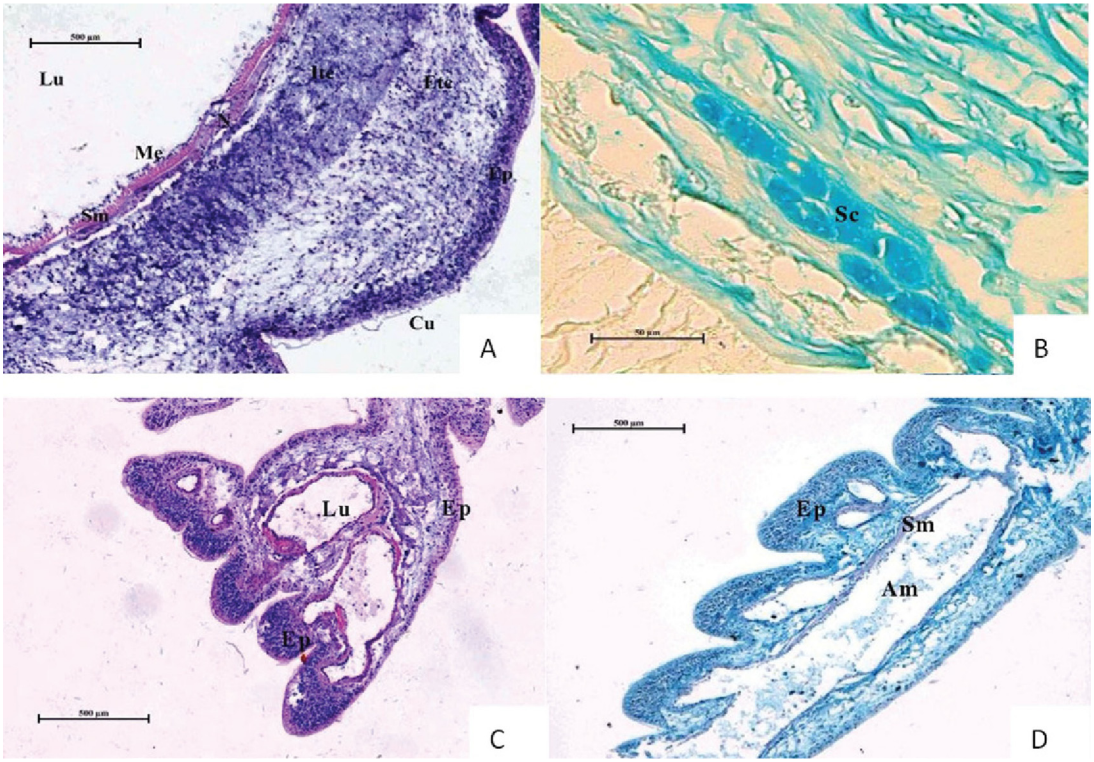

FIGURE 4.20

Histology of tentacle in *A. japonicus.* (A) Cross-section of tentacle petiole (hematoxylin–eosin staining, HE), scale bar = 500 µm; (B) cross-section of tentacle petiole (alcian blue and periodic acid Schiff staining, AB-PAS), scale bar = 50 µm; (C) cross-section of the end of a scutiform tentacle (HE), scale bar = 500 µm; (D) vertical section of the end of a scutiform tentacle (AB-PAS), scale bar = 500 µm. Am, mucous; Cu, cuticle; Ep, epithelial layer; Etc, loose connective tissue; Itc, dense connective tissue; N, nervous layer; Sm, muscular layer; Me, body cavity lining; Sc, mucous cells; Lu, water vascular cavity.

From Zhao, 2010

containing acid mucopolysaccharide (Figure 4.20B). The end of the scutiform tentacle consists of a cuticle, epithelial layer, connective tissue, nervous layer, muscular layer, and body cavity lining (Figure 4.20C, D; Zhao, 2010).

Five radial canals branch off from the ring canal. There are two radial canals on the dorsal side of the body and three on the ventral side. The five radial canals extend to the end of the body along the central sulcus of the five longitudinal muscles (Sui, 1988). Each radial canal divides into a number of lateral branches. The ventral lateral branches connect with the ambulacral tube feet, and the dorsal ones connect with papillae (Sui, 1988). An ampulla is located at the end of each ambulacral tube foot, and its muscles have strong contractile abilities. There are valves in the ampulla which can help seawater enter the ambulacral tube feet with the contraction of the ampulla (Sui, 1988).

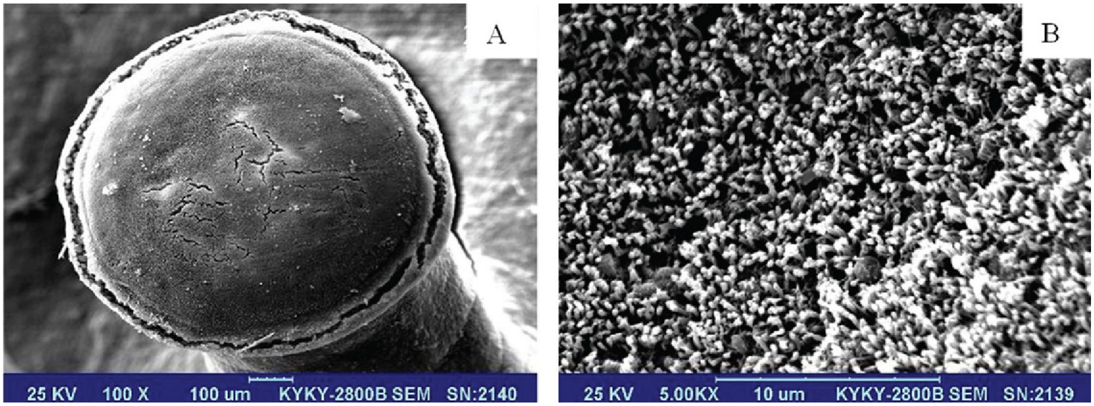

FIGURE 4.21

The ambulacral foot of *A. japonicus* (scanning electron microscopy). (A) Surface of ambulacral foot; (B) microvilli on the surface of the adhesive disc.

From Zhao, 2010

The ambulacral tube foot consists of the petiole and the adhesive disc. The latter can be divided into a peripheral zone and a central depression (Zhao, 2010). The surface of the adhesive disc is densely covered by a mass of microvilli and ostioles (Zhao, 2010; Figure 4.21).

4.9 HEMAL SYSTEM

The hemal vascular system of *A. japonicus* is mainly composed of ventral and dorsal sinuses found along the digestive tract (Zhu, 2009). The ventral sinus extends from the pharynx to the end of the posterior intestine. In the middle of the anterior intestine and middle intestine, a horizontal branch connects the ventral sinus to both sections of the intestine (Liao, 1997; Zhu, 2009).

The dorsal sinus starts from the end of the anterior intestine and runs along the middle intestine. A reticular formation, composed of many parallel minute vessels, occurs at the end of the anterior intestine. This reticular formation connects the dorsal sinus and collecting vessel to the alimentary canal. The extended minute vessels on the middle intestine turn into the rete mirabile and intertwine together with the secondary branches of the left respiratory tree. The dorsal sinuses turn into a tube at the posterior intestine, which extends to the end of the digestive tract (Liao, 1997; Zhu, 2009; Figure 4.22A).

The dorsal and ventral sinuses of *A. japonicus* may be more closely connected with the digestive tract through small vessels in the intestinal wall (Zhu, 2009; Figure 4.23B). Many sinuses extend through the connective tissue into the intestinal wall (Zhu, 2009; Figure 4.22C).

4.10 REPRODUCTIVE SYSTEM

A. japonicus is dioecious, although it is difficult to distinguish males and females based on external morphology. The reproductive system consists of a single gonad, composed of a cluster of tubules emptying into a single duct, which opens on the dorsal surface, close to the tentacles.

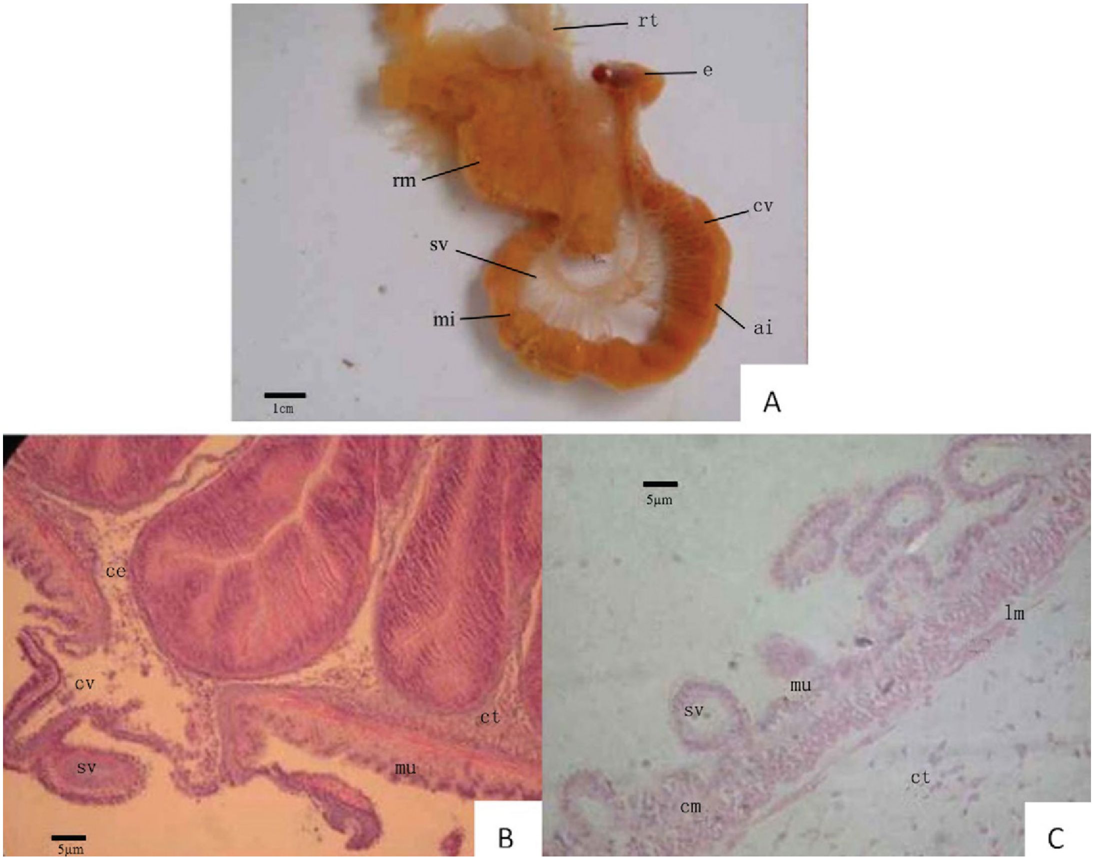

FIGURE 4.22

Hemal system of *A. japonicus.* (A) Viscera; (B) histology of the collecting vessel of the dorsal sinus; (C) transversal section of the intestinal wall. ct, connective tissue; ds, dorsal sinus; vs, ventral sinus; e, esophagus; ai, anterior intestine; mi, middle intestine; t, respiratory tree; sv, small vessel; cv, collecting vessel; rm, rete mirabile; mu, mucosa; cm, circular muscle; lm, longitudinal muscle.

From Zhu, 2009

The gonad is a dendritic tubule attached to the dorsal mesentery. It consists of 11–13 main branches with several secondary branches. Some of the branches can reach a length of 20–30 cm or longer during the reproductive season (see Chapter 6 for details on the reproductive biology). These main branches converge into a gonoduct that opens into a gonopore through the body wall (Sui, 1988). The gonopore of *A. japonicus* is located in the interambulacrum on the dorsal side of the anterior end. The melanin pigmentation around the gonopore increases as the gonad develops. The gonopore is circular and has a diameter of about 4–5 mm. It is found in the middle of a genital papilla. During the reproductive season, the female gonad, or ovary, is orange (Figure 4.23A) and the male gonad, or testis, is milky white (Figure 4.23B). In the nonreproductive season, the gonad is tiny and it is generally difficult to distinguish genders from its color.

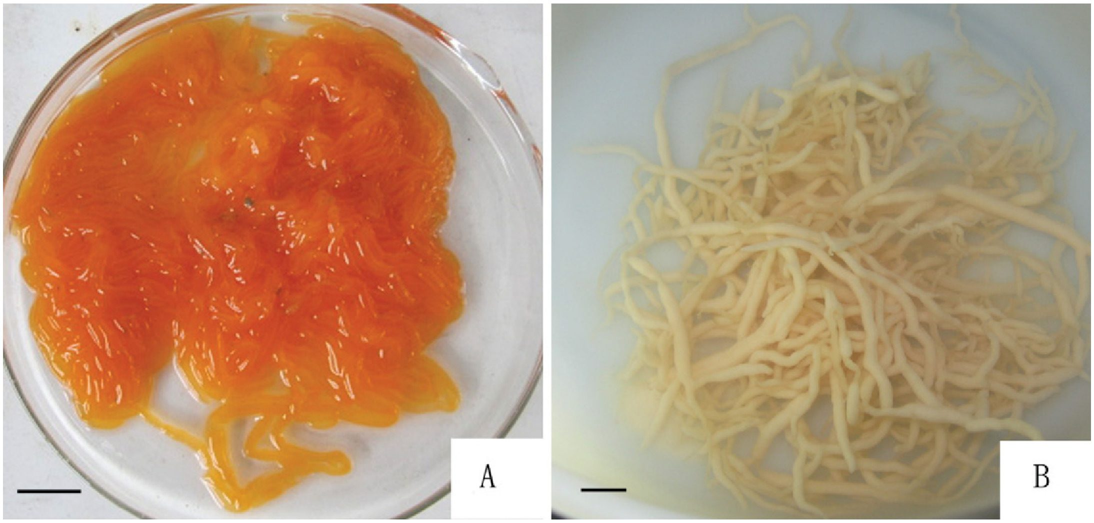

FIGURE 4.23

Gonad of *A. japonicus.* (A) Mature gonad tubules of female; (B) mature gonad tubules of male. Scale bar = 1 cm.

A, photo by Jie Tan; B, photo by Shilin Liu

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CHAPTER

SPATIAL DISTRIBUTION, POPULATION STRUCTURES, MANAGEMENT, AND CONSERVATION

5

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SUMMARY

The sea cucumber *Apostichopus japonicus* is one of the most important commercial species in Asian countries including China, Japan, Russia, Republic of Korea, and Democratic People's Republic of Korea. Catches of wild *A. japonicus* have significantly declined over the past years throughout its distribution range, and this species is now being massively produced through hatcheries and sea ranching. This chapter introduces the larval, juvenile, and adult spatial distributions, population structures, and dynamics as well as some data on fishery management and conservation.

Keywords: *Apostichopus japonicus*; conservation; fishery management; population structure; sea cucumber; spatial distribution

5.1 SPATIAL DISTRIBUTION

5.1.1 ABUNDANCE AND POPULATION DYNAMICS OF LARVAE

The appearance of *A. japonicus* larvae coincides with its spawning season, which is closely related to annual fluctuations in seawater temperature at the various sites (see Chapters 6 and 7). Studies in Busse Lagoon (Aniva Bay, Sakhalin Island, Russia) and in Novgorodskaya Bight (Posyet Bay, Sea of Japan) showed that larvae of *A. japonicus* appear in low density in the first half of August. By mid-August, larval density has increased from 250 to 1131 ind. m^{-3}. By the end of August the density has decreased sharply, and in early September, only a small number or even no sea cucumber larvae can be found (Kulikova and Sergeenko, 2003). Studies also showed that *A. japonicus* larvae are absent from plankton samples in many survey stations, which may be related to the behavior of different early life stages (blastula, gastrula, auricularia, dolidaria) in conjunction with a complex system of currents in the study sites. It has been found that embryos (blastula, gastrula) of sea cucumber tend to occur near the water surface and, as they develop, larvae move closer to the sea floor (Kashenko, 1998). Therefore, they cannot always be captured with a plankton net. Abundances of *A. japonicus* larvae are low in some years, which can be explained by the existence of unfavorable conditions affecting reproduction. For example, in an unfavorable year, maximum larval density recorded in Posyet Bay was between 2 and 80 ind. m^{-3} (Mokretsova, 1990).

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http://dx.doi.org/10.1016/B978-0-12-799953-1.00005-2

5.1.2 HORIZONTAL DISTRIBUTION OF JUVENILES AND ADULTS

A. japonicus is widely distributed throughout the northwest Pacific, including the Bohai Sea, the Yellow Sea, the east coast of Russia, the coast of Japan, Democratic People's Republic of Korea, and Republic of Korea (Liao, 1997) (see Chapter 3 for map of geographic distribution). Generally, pebbles, gravel, sand and muddy-sand areas close to the coastline, inner bay reefs, and macrophyte habitats unaffected by freshwater runoff constitute good habitats for *A. japonicus* (Levin, 1979). The most important elements determining the survival, abundance, and distribution of *A. japonicus* are water temperature, salinity, bottom type, as well as habitat for the larvae to settle. Populations can also be large in zones with strong currents (10–30 cm s^{-1}) and high level of dissolved oxygen (Chen, 2004) (see below for details on the various parameters).

5.1.2.1 Importance of salinity

Salinity is an important environmental factor affecting the distribution and survival of *A. japonicus*. Sang (1962) found evidence that salinity is lower in surface waters than in deep waters in Mikawa Bay (Japan) during the spawning period. He suggested that the larval settling in the shallow waters along the coasts could therefore be affected by dramatic salinity fluctuations. Sang (1962) also suggested that during the spawning season, low salinity in shallow water habitats plays an important role in determining the final distribution of *A. japonicus*. The salinity suited to *A. japonicus* is between 24.2 and 34.6. By conducting salinity gradient experiments, Sang (1962) found that in low salinity seawater (<18.1), the epidermis of the body wall turned white and shriveled, and that all animals died after a few days of exposure.

5.1.2.2 Importance of temperature

Temperature is another important environmental factor affecting the distribution and physiology of *A. japonicus*. Along the coast of Japan, the water temperature fluctuates between 13 and 22 °C during the spawning season. Generally, the temperature is 13–16 °C at the onset and 18–22 °C at the end of the spawning season (Sang, 1962). Water temperature is the most direct factor causing the sea cucumber to enter aestivation, which is a particular phenomenon observed during the warm season (see Chapter 11 for details on aestivation). The water temperature threshold at which *A. japonicus* goes into aestivation is around 24.5 °C. Those individuals found between the intertidal and the shallow subtidal zone, which can be exposed to these high temperatures, will migrate to deeper water and hide beneath reefs/rocks or crevices of the sea floor until the temperature decreases.

5.1.2.3 Nursery habitats

The distribution of juvenile sea cucumbers is limited to the shallow waters near the coast. As Mitsukuri (1903) already stated, the juveniles of *A. japonicus* attach to the underside of rocks in the intertidal zone, to macrophyte blades or to the leaves of seagrass along the coast of Kanagawa Prefecture (Japan). To settle and survive, the larvae of *A. japonicus* need rocky bottoms and macrophyte fields, which provide attachment and food to newly settled pentactulae and early juveniles.

5.1.3 SIZE SEGREGATION WITH DEPTH

Vertically, the habitat of *A. japonicus* extends from the intertidal zone to depths of 15–20 m. Selin (2001) studied the vertical distribution and aggregations of *A. japonicus* in Vostok Bay, Sea of Japan (Russia),

during early September 2000 and the results showed that the highest density (8.3 ± 0.5 ind. m^{-2}) and biomass (131.88 ± 10.50 g m^{-2}) of sea cucumber was around 0.5–1.5 m. As depth increased, both biomass and density of *A. japonicus* decreased by a factor of 1.5–3 at 5–6 m and a factor of 220–830 at 8–15 m. The aggregations also vary at various depths with respect to their size–age composition; large individuals tend to inhabit deeper waters and small individuals shallower waters (Selin, 2001). In Mikawa Bay (Japan), a similar size distribution of *A. japonicus* was observed with small individuals (50 g or less) found in the shallow-water habitats along the coast, those between 50 and 100 g at a depth of 5 m or less, those between 100 and 150 g around 5–10 m depth, and the largest sea cucumbers (200 g or more) distributed at depths down to 15 m or more (Sang, 1962). The type of bottom sediments was closely related to depth, with muddy sediment becoming more common at greater depth. Consequently, as depth increases, more organic carbon and nitrogen are available, which may explain the size distribution of sea cucumbers (Sang, 1962).

5.1.4 SEASONAL DISTRIBUTION

Yamana et al. (2009) studied the seasonal distribution pattern of adult *A. japonicus* in the subtidal zone of Yoshimi Bay, Japan, over a two-year period. Their results showed a clear seasonal change in the distribution pattern over time; most individuals were found directly on the seabed or on adjacent structures during winter and spring, whereas they were found on the wharf structures during summer and autumn (Figure 5.1). Such changes were considered to be due to the migration of individuals within the study site. A trend was also reported by Hamano et al. (1989) in the Seto Inland Sea, Japan, where sea cucumbers were difficult to find during summer, which corresponds to aestivation in deeper waters. Similarly, Dubrovskii and Sergeenko (2002) observed that *A. japonicus* in the Kunashir Island, Southern Kurile Islands (Russia) hid in crevices of shell clumps of *Crassostrea gigas* and under various surfaces for aestivation, at which time they are difficult to find.

5.2 POPULATION STRUCTURE

5.2.1 POPULATION DENSITY

An *A. japonicus* population density of 0.12–0.34 ind. m^{-2} was observed for depths between 1 and 20 m in Kievka Bay in the Sea of Japan, Russia (Gavrilova and Sukhin, 2011). Similar values of 0.17 and 0.4 ind. m^{-2} were reported by Maslennikov (1894) in Ussuri Bay, Russia and by Zaks (1930) in the Far Eastern Seas, Russia. In Vostok Bay, in the Sea of Japan, Russia, the average population density was 0.5 ind. m^{-2}, which was relatively stable for a period of 10 years (Selin and Chernyaev, 1994). A population density of 0.024 ind. m^{-2} was reported in Peter the Great Bay in the Sea of Japan (Gavrilova and Sukhin, 2011). It was estimated that in the fishing grounds of Ehima, Atsumi peninsula of Japan, the natural density in 1956 was 0.007–0.193 ind. m^{-2} (Sang, 1962). Therefore, the range of 0.1–0.5 ind. m^{-2} may be considered the average population density for this species. Interestingly, in Taozi Gulf of Yantai, China, the estimated population density was 1.05 ind. m^{-2} (Dong and Zhou, 1984), corresponding to much higher values than the average, although this natural population has now been completely disseminated by overfishing.

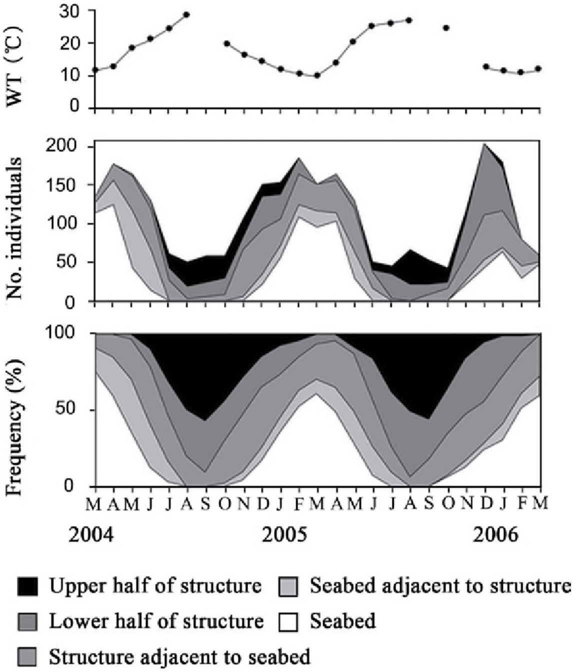

FIGURE 5.1

Monthly changes in numbers and frequencies of *A. japonicus* individuals found in Yoshimi Bay, Japan. WT in *Y* axis refers to water temperature.

From Yamana et al., 2009

5.2.2 SEX RATIO

Because of indistinctive secondary sexual characteristics (morphological dimorphism) between males and females of *A. japonicus*, there are relatively few research studies on field sex ratio. Sex ratio was recorded in investigations on biological characteristics of adults of *A. japonicus* from different geographic populations. The reported female–male ratio is 1:1.03 for a population in Taozi Gulf of Yantai, China (Dong and Zhou, 1984). In a coastal population near Dalian, China, the sex ratio was 1:1.34 (Wang, 2009). Similar results were obtained by Sang (1962) who examined 213 individuals around the coast of Japan and found a female–male ratio of 1:1.09.

5.2.3 POPULATION SIZE

Most studies on population size of *A. japonicus* were carried out on regional scales. Sang (1962) reported that the population in Mikawa Bay (Japan) was about 630,000 individuals with a biomass of 6,600 kg (wet weight). In Kievka Bay, Sea of Japan (Russia), there were about 200,000 individuals on a surface area of 80 ha (Gavrilova and Sukhin, 2011). Dong and Zhou (1984) showed that the biomass of *A. japonicus* in Taozi Gulf, at Fushan, Yantai (China) was about 400,000 kg (wet weight) on 340 ha.

Due to over-exploitation, natural populations of *A. japonicus* have declined sharply in China and no studies on current natural biomass have been conducted.

5.2.4 BODY LENGTH–WEIGHT RELATIONSHIP

Dong and Zhou (1984) showed that for *A. japonicus* with body lengths (L) ranging from 6 to 30 cm, equivalent to body weights (W) from 4 to 257 g (wet weight), there was a power function relationship following the equation $W = 0.0649L^{2.854}$. Sang (1962) showed the correlation between total body weight and body length in an index curve (Table 5.1). Inflection points in two locations along the curve around 6.0 cm and 16.0 cm were detected, which could be related to reproduction, aestivation, and growth.

5.2.5 POPULATION GENETICS

Kanno et al. (2006) analyzed the genetic relationship among three sympatric color variants (red, black, green) of *A. japonicus* in populations around Japan, using 11 microsatellite loci (see Chapter 3 for a description of color variants in *A. japonicus*). Genetic differentiation test among the three sympatric color variants showed that the red type is different from the other two (black, green). Phylogenetic trees constructed from different localities (Miyagi, Oita, Aomori, Hokkaido in Japan and Qingdao in China) showed two distinct clusters, one for the red types and the other for the green and black types. In addition, the sympatric green and black variants formed one subcluster with strong bootstrap support at each locality. These results indicate the separate status of red versus the other color types, and also support the population identity of sympatric green and black types, which provides a clear guide for current management of these natural populations.

Kim et al. (2008) examined the population genetic characteristics of *A. japonicus* in Republic of Korea using microsatellite markers. The phylogenetic tree revealed two distinct clusters. One cluster was formed by the eastern population (Gangneung) and a second cluster consisted of the subpopulations of the western and southern populations (Gomsoman, Taean, Yeosu, and Geoje). Moreover, Chang et al. (2009) found genetic differentiation among five *A. japonicus* populations collected from China, Russia, Japan, and Korea using 10 microsatellite loci. In this study, a large genetic distance was observed between two natural populations collected at the same site from Aomori, Japan, which indicated that

Table 5.1 Relationship Between the Body Length (*L*) and Total Body Weight (TW) for the Sea Cucumber *A. japonicus*.

	Log TW = log a + (b log e)L	
Body Length (cm)	**log a**	**b log e**
≤6.0	−0.4434	0.2729
6.1–16.0	0.5051	0.1189
≥16.1	1.4453	0.0591

Modified from Sang, 1962

some sea cucumbers might have immigrated from Far East Russia to Japan in the last glacial period favoring the speciation of the Japanese population of the red variant. Kang et al. (2011) analyzed populations of the green type from China (Jiaonan, Wendeng, Rongcheng, Changdo, and Dalian on the Yellow Sea coast of northern China) and Republic of Korea (Taean, Heuksan, Geomun, Geoje, Pohang, and Ulleung around the Korean Peninsula) and of the red type from Republic of Korea (Pohang, Ulleung, and Dokdo) using nine microsatellite loci. Results showed no differences between the green types from Korea and China, whereas the differences between the green and red types were significant. The information obtained in the study demonstrated the feasibility of microsatellite analysis for discrimination between color variants, which will be useful for the development of diagnostic kits for the discrimination of red and green types before seed release for stock restoration.

Genetic maps of the sea cucumber *A. japonicus* were constructed based on AFLP, microsatellite and SNP markers (Li et al., 2009; Yan et al., 2013), which paves the way for the development of a high-resolution genetic map and mapping of the functional genes and quantitative trait loci for further application of a marker-assisted selection breeding strategy in this species.

5.3 FISHERIES MANAGEMENT AND CONSERVATION

5.3.1 FISHERIES AND FISHING METHODS

Wild capture fisheries of *A. japonicus* in China have declined drastically in Shandong and Liaoning provinces, which account for most of China's production. Wild captured *A. japonicus* in these and surrounding areas declined from 130–140 tons in the 1950s to values between 26 and 40 tons in the 1970s (Choo, 2008). By the end of the 1970s, capture of wild *A. japonicus* had dropped to almost zero and current landings of wild caught *A. japonicus* in China are considered nonexistent (Chen, 2004). In China, the total landing of *A. japonicus* from farming reached over 5800 tons (dry weight) in 2002 (Chen, 2004) (also see Chapters 2, 16, and 21). Konstantinova (2004) noted that the sea cucumber (species not identified in the article, but likely to be *A. japonicus*) resources in Primorsky Krai, located in the extreme southeastern region of the Russian Federation, have decreased to 16–20% of the level of the stock that existed in the 1960s. Most of the wild *A. japonicus* specimens in Russia currently weigh around 40–60 g (wet weight), which is below the marketable size (Choo, 2008). In the Democratic People's Republic of Korea, *A. japonicus* is commonly captured for food and it is reported to be severely exploited (Choo, 2008) (see Chapter 24). Average annual capture production of *A. japonicus* from the Republic of Korea was about 1902 tons from 1990 to 1999, and 1120 tons from 2000 to 2005, representing a decline of about 40% over 10–15 years (Choo, 2008) (see Chapter 23). In Japan, the catches of *A. japonicus* have decreased at least 30% over the past 30 years, dropping from over 10,000 tons (wet weight) in 1978 to 7,133 tons in 1987 (Bruckner et al., 2003). There seems to be a stable trend with a range of landings for *A. japonicus* between 7000–9000 tons per year, between 2000 and 2005 (Choo, 2008) (see Chapter 22).

Traditional fishing gear, such as small bottom trawl nets, spears, hooks, and scoop nets and diving have been developed to fit the sea cucumber fishing traditions in various areas (Arakawa, 1990) as shown in Figure 5.2. Soaring market prices have triggered the development of aquaculture and sea ranching of *A. japonicus*. As early as the 1950s, artificially produced seeds of sea cucumber were tested (Zhang, 1958). However, the main progress was only achieved in the 1980s (Sui, 1985; Zhang and Liu, 1998).

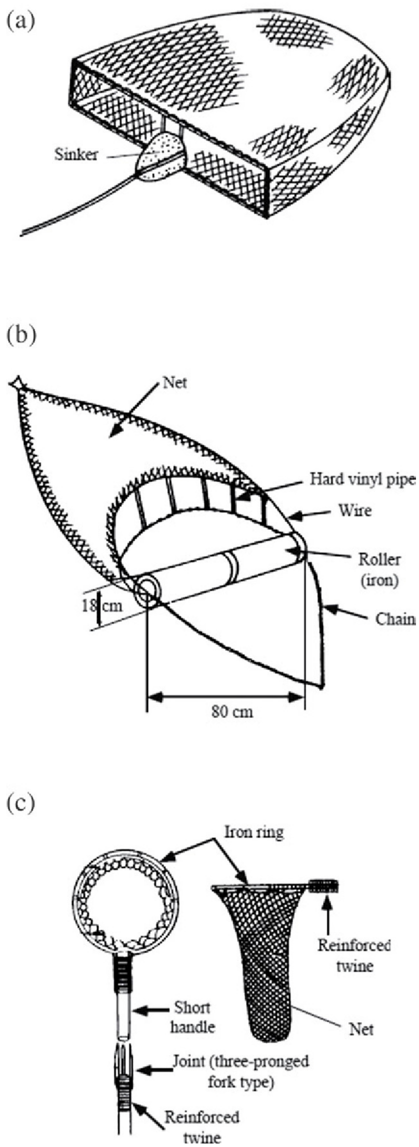

FIGURE 5.2

Traditional fishing gear used to capture the sea cucumber *A. japonicus* in Japan. (a) Beam trawl net, (b) roller-pulling net, and (c) scoop net.

Adapted from Arakawa, 1990

5.3.2 MANAGEMENT AND CONSERVATION

The consumption of dried sea cucumber in China can trace its history back to the late Ming and early Qing dynasties (see Chapters 1 and 2). The cross-border trade has also been closely connected to the neighboring countries, such as Japan, Republic of Korea, and Democratic People's Republic of Korea for *A. japonicus* and Southeast Asia and Indo-Pacific islands for the tropical species (Akamine, 2007). Nowadays, increasing demand and overfishing have led to major concerns over resource conservation and have also triggered conflicts between environmentalists and sea cucumber fishermen, for example, during the so called "sea cucumber war" concerning another species of Stichopodidae (*Isostichopus fuscus*) in the Galapagos Islands (Akamine, 2010; Stutz, 1995).

Japan was the first country in Asia to develop conservation measures for the sea cucumber fisheries, including limiting total annual catch, regulating the minimum harvest size and mesh size, and closing certain areas or seasons to fishing in order to promote reproduction (Choo, 2008; see Chapter 22). According to the Fisheries Law in Japan, no-one can freely collect and gather holothurians without a fishing right, as the animal is designated a common fishery right species. The prefectural governor, together with the Sea-area Fishery Adjustment Commission, plays an important role in resource management, especially for holothurian fisheries in Japan (Akamine, 2004; see Chapter 22). During the 1980s and 1990s, management of sea cucumber was actively practiced and implemented in Japan. For example, in Rishiri Island, the harvestable weight of sea cucumber was raised to 130 g in 1990 and animals less than 10 cm in length were sorted out for release back to the sea. In the interest of conserving resources, a self-regulatory 50-ton limit of total catch was also set for Rishiri Island (Akamine, 2010).

Closed fishing seasons (March to November in Japan) were determined according to spawning season and seasons of high water temperatures under local fishing regulations (Arakawa, 1990). These measures, combined with the release of seedlings, were determined to be very effective in preserving the resources. There is an example of a recovery plan in Oura Bay of Saga Prefecture in Kyushu, Japan. After releasing 1700 juveniles in an area of 700 m^2, fishing was prohibited for two years in an area of 1500 m^2 encompassing the area of release. Total catches were thereafter approximately 30 times higher than before (Arakawa, 1990). Similarly, fishing adult sea cucumbers was prohibited for two years in an area of 1938 m^2 in Miyagi Prefecture, leading to an increase in total catches by 2.5 to 3.7 fold (Arakawa, 1990).

In China, a national conservation zone for *A. japonicus* has been established in Liaoning Province although many such zones existed at the county and village levels in Liaoning and Shandong provinces (Chen, 2004).

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CHAPTER

REPRODUCTIVE BIOLOGY

6

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SUMMARY

Knowledge of the reproductive biology of a species is instrumental in designing breeding and aquaculture programs, and ultimately restoring and enhancing wild stocks in the pursuit of sustainable exploitation. This chapter presents the reproductive biology of the sea cucumber *Apostichopus japonicus*, including its gonad development, sex ratio, size at first sexual maturity, and fecundity. The characterization of different gonad morphologies and corresponding gametogenesis stages is outlined. We also cover the regulation of oocyte maturation and associated biochemical and molecular processes, as well as correlations between environmental factors and reproduction.

Keywords: *Apostichopus japonicus*; gametogenesis; gonad morphology; oocyte maturation; reproductive cycle; spawning

6.1 ANATOMY OF THE REPRODUCTIVE SYSTEM

Like most sea cucumbers, *Apostichopus japonicus* is dioecious, although it is virtually impossible to differentiate males from females based on their external appearances. Unlike other echinoderms (e.g., sea stars, sea urchins, brittle stars, and crinoids), the gonad of *A. japonicus* is not radially symmetric (Liao, 1997). Rather, it consists of numerous branched tubules that form two clusters on either side of the dorsal mesentery in the interambulacral region of the anterior coelom. The reproductive tubules (or gonad tubules) converge to the anterior part of the body and form a gonoduct. The latter opens externally through a gonopore, located on the dorsal surface of the body wall, behind the mouth and the tentacles.

In *A. japonicus*, the gonad wall consists of a coelomic epithelium, a muscular layer, a connective tissue layer, and a germinal epithelium that lines the lumen of the tubule. The muscular layer includes circular and longitudinal muscle fibers. The sexual cells originate from the germinal epithelium; accessory cells (somatic cells) are typically present in the inner epithelium. The gonoduct consists of a ciliated epithelium encompassed by connective tissues and attached to the dorsal mesentery (Liao, 1997). Chapter 4 provides further details on the anatomy of the reproductive system.

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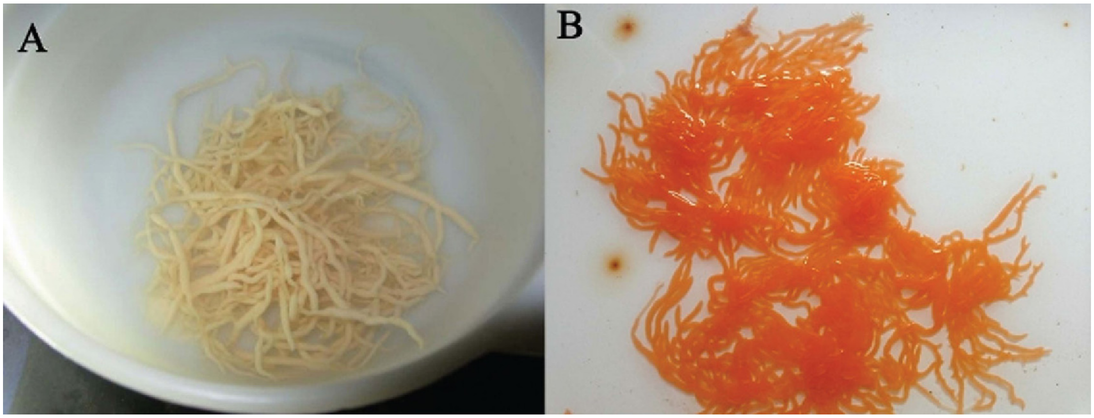

FIGURE 6.1

Freshly extracted gonads of *A. japonicus.* (A) Mature gonad of male. (B) Mature gonad of female.

Photo A by Shilin Liu, Photo B by Qiang Xu

The gonad tubules become enlarged (swollen) and branched when the gonad is sexually mature. The length of the whole gonad is only 2 cm in October (resting stage), but it can reach 25–35 cm in July (mature stage). At maturity, the entire gonad of the male (testis) is glossy white (Figure 6.1A), whereas the gonad of the female (ovary) is orange and slightly translucent (Figure 6.1B) (Liao, 1997). Additional details on gonad maturation relative to broodstock conditioning and spawning can be found in Chapter 7.

6.2 GONAD DEVELOPMENT

6.2.1 GAMETOGENIC STAGES

Histology of the gonad in *A. japonicus* has been used to divide the reproductive cycle into five major developmental stages. A description of the histological features of each gametogenic stage is provided next (Tanaka, 1958; Sui et al., 1985).

6.2.1.1 Resting stage

In both sexes, the gonad wall is distinctly shrunken and no visible fold along the germinal epithelium can be detected. In females, the gonad tubules are small and unbranched, the germinal epithelium usually exhibits a single layer, or sometimes two layers of oogonia or/and harbors few previtellogenic oocytes with diameters of ~10 μm (Figure 6.2, Photo 1; Tanaka, 1958). In males, the gonad has many unbranched small tubules and the germinal epithelium consists of 1–3 spermatogonia or spermatocyte layers, distributed along the inner side of the follicle (Figure 6.2, Photo 6; Tanaka, 1958; Liao, 1997).

6.2.1.2 Early growth stage

The germinal epithelium becomes convoluted. A layer of oocytes measuring 30–50 μm in diameter is distributed along the female germinal epithelium. There is no obvious size difference among the

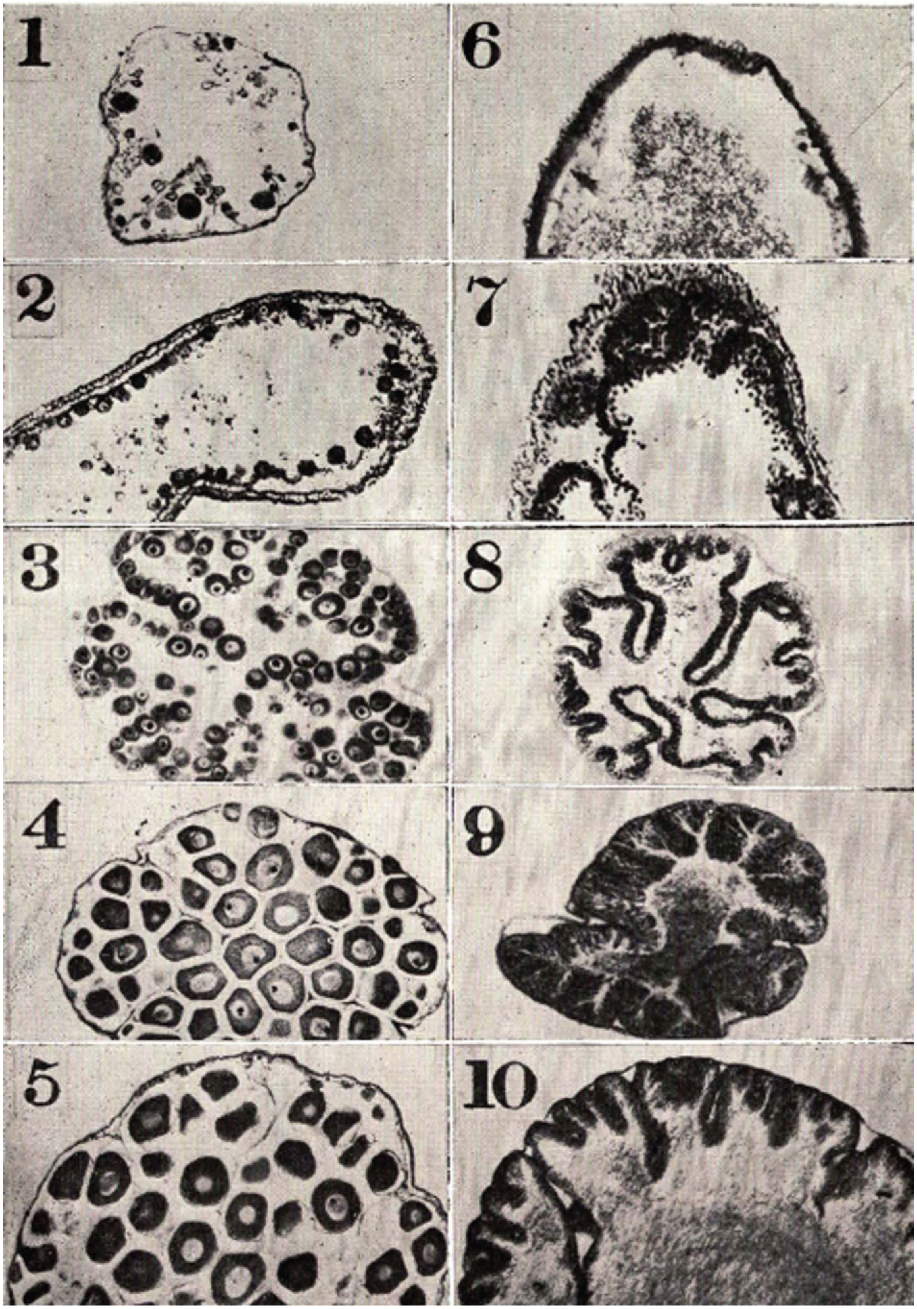

FIGURE 6.2

Gonad development stages in *A. japonicus.* (1) Female gonad, resting stage, ×54; (2) female gonad, early growth stage, ×90; (3) female gonad, growth stage, ×72; (4) female gonad, mature stage, ×54; (5) female gonad, spent stage, ×54; (6) male gonad, resting stage, ×80; (7) male gonad, early growth stage, ×72; (8) male gonad, growth stage, ×72; (9) male gonad, mature stage, ×54; (10) male gonad, spent stage, ×54.

From Tanaka, 1958

oocytes, but a deeper investigation reveals that oocytes closer to the germinal epithelium are slightly smaller compared to those found further away in the lumen of the tubule. All oocytes are surrounded by follicle cells and have a large germinal vesicle with a nucleolus (Figure 6.2, Photo 2; Tanaka, 1958). In males, the germinal epithelium consists of 1–2 spermatocyte layer(s) and spermatozoa are not yet formed (Figure 6.2, Photo 7; Tanaka, 1958; Liao, 1997).

6.2.1.3 Growth stage

The ovarian wall becomes thick and the developing oocytes are connected to the ovarian stroma. Oocytes grow rapidly to reach a diameter of 60–90 μm, and they begin to fill up most of the lumen (Figure 6.2, Photo 3; Tanaka, 1958). Folds are becoming visible along cross-sections of the male gonad tubules. The number of spermatocytes increases markedly in several layers along the germinal epithelium. There are a few spermatids along the germinal epithelium and spermatozoa are visible in the lumen of the tubules (Figure 6.2, Photo 8; Tanaka, 1958; Liao, 1997).

6.2.1.4 Mature stage

The gonad reaches its maximum size in both sexes and the gonad wall reaches its minimal thickness. The branches of the tubules also become more numerous and swollen. The oocytes fully occupy the gonad tubules and measure about 130 μm in diameter (Figure 6.2, Photo 4; Tanaka, 1958). The lumens of the male tubules are densely packed with spermatozoa. The presence of a few remaining spermatocytes is also observed along the germinal epithelium (Figure 6.2, Photo 9; Tanaka, 1958; Liao, 1997).

6.2.1.5 Spent stage

The ovarian wall becomes thick and wrinkled (as the tubule shrinks in size). Relict oocytes are occasionally present in the lumen of largely empty tubules (Figure 6.2, Photo 5; Tanaka, 1958). The male tubules have an empty lumen except for a few unreleased spermatozoa. However, the germinal epithelium is still lined with many spermatocytes (Figure 6.2, Photo 10). A few phagocytes (diameter of 6–7 μm), which have many granules, are scattered in the lumen of the gonad tubules of both sexes (Tanaka, 1958; Liao, 1997).

6.2.2 MICROSTRUCTURAL AND ULTRASTRUCTURAL FEATURES

The gonad of juvenile *A. japonicus* (0.1 cm in length) contains about 10 primordial germ cells (Figure 6.3A, B; Yan et al., 2011). The primary structure of the gonad, which forms as the coelomic cavity grows in juveniles, reaching 0.5 cm in length (Figure 6.3C; Yan et al., 2011). When the juvenile reaches a length of 3 cm, the gonad is structurally similar to that of the adult, with two branches and a wall composed of two main layers: the outer coelomic layer consists of smaller epithelial cells and the inner layer consists of loosely arranged germ cells (Figure 6.3D, E; Yan et al., 2011). In 4-cm-long juveniles, part of the inner layer of the gonad wall folds inward to form germ cell-based ridge-like protrusions, which become more developed as the juveniles continue to grow (Figure 6.3F; Yan et al., 2011).

During the spermatogenesis of *A. japonicus*, the spermatogonium has a distinct nucleolus, and some proacrosomal granules. Chromatin condensation is observed during the primary spermatocyte phase. The flagellum emerges in the following spermatid phase. The proacrosomal vesicle forms and gradually develops into an acrosome. Mitochondria gradually fuse and increase in size. The mature spermatozoon belongs to the primitive type, consisting of a head, middle piece, and flagellum. The

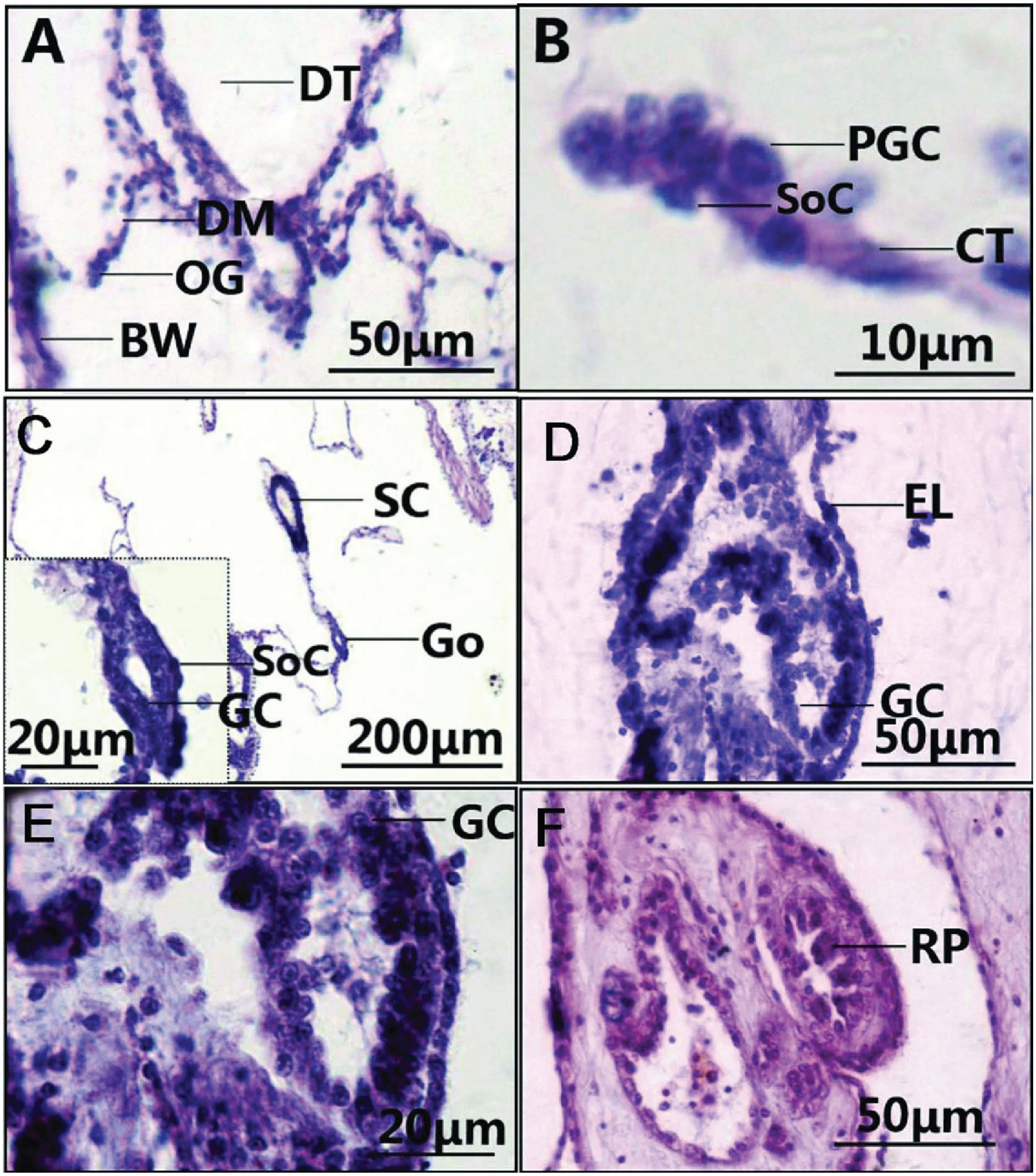

FIGURE 6.3

Genesis and early differentiation of gonad in *A. japonicus.* (A) Cross-section of 0.1-cm-long juvenile through original gonad; (B) magnification of the gonad in 0.1-cm-long juvenile; (C) longitudinal section of 0.5-cm-long juvenile through gonad (inset showing magnification); (D) longitudinal section of 3-cm-long juvenile through gonad; (E) magnification of D; (F) longitudinal section of 4-cm-long juvenile through gonad. BW, body wall; CT, connective tissue; DM, dorsal mesentery; DT, digestive tract; EL, epithelioid layer; GC, germ cell; Go, gonad; OG, original gonad; PGC, primordial germ cell; RP, ridge-like protrusions; SC, stone canal; SoC, somatic cell.

From Yan et al., 2011

acrosome is spherical with a diameter of about 3 μm (head area), and the flagellum is about 50–60 μm in length. There is only one mitochondrion in the mature spermatozoon. The flagellum exhibits a simple "9+2" type of microtubule structure (Pang et al., 2006). Accessory cells are found in the inner gonad epithelium of male *A. japonicus*. These cells have no definite shape, appearing amorphous, and covering the basal lamina in a thin layer. The inner surface of the basal lamina is almost completely covered by this layer of accessory cells. Each accessory cell has a widened section containing the nucleus and other organelles (Reunov et al., 2001).

6.3 REPRODUCTIVE CYCLE

The reproductive cycle of *A. japonicus* is annual. The dynamic of gonad development varies, mainly depending on environmental factors, such as seawater temperature and other parameters that fluctuate across the different geographical areas of its distribution range.

6.3.1 SEASONAL CHANGES IN GAMETOGENIC STAGES AND GONAD INDEX

The gonad is in the growth stage from May to July in the northern Yellow Sea (China), at which time the gonad index (ratio of gonad wet weight to body wall wet weight) increases from its lowest values. In mid-July, the gonad becomes mature and the gonad index peaks. It subsequently decreases to reach its lowest level in late September. Therefore, the spawning season of *A. japonicus* lasts from mid-July to mid-September (Figure 6.4; Zhou et al., 2001). The seasonal patterns of gonad index are somewhat mirrored in the two sexes (Figure 6.4; Zhou et al., 2001). However, the gonad index values of males increase more quickly and reach a peak earlier than that of females. Similarly, the gonad index of males decreases more quickly than that of females. In mid-August, the male gonad index drops to below 2.3%, whereas the female gonad index remains above 9.7%, which might suggest that the spawning of male populations is brief relative to the female's, which spawn over a longer period (Figure 6.4; Zhou

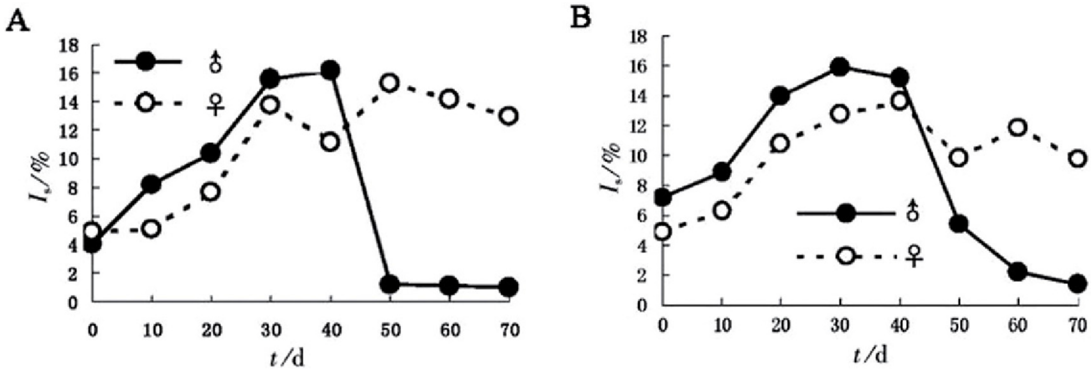

FIGURE 6.4

Variation in the gonad index of *A. japonicus*. (A) Gonad index of from June to August 1991 (northern Yellow Sea, China). (B) Gonad index between June and August 1992. I_s, gonad index; t, time; d, day; ♂, male; ♀, female.

From Zhou et al., 2001

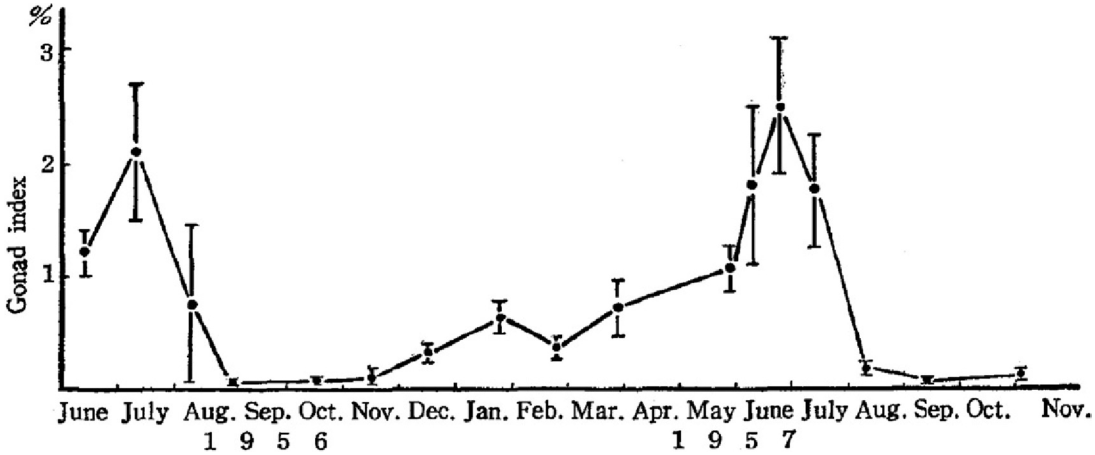

FIGURE 6.5

Seasonal changes in the gonad index of *A. japonicus* in southern Hokkaido, Japan. Vertical lines correspond to standard errors.

From Tanaka, 1958

et al., 2001; see Chapter 7 for additional information on spawning). Variations in gonad indices must always be interpreted very cautiously, as they do not only reflect gamete maturation and therefore are not a perfect indication of spawning events (Mercier and Hamel, 2009).

In southern Hokkaido (Japan), the gonad index rises very rapidly from June to reach a peak in mid-July, when *A. japonicus* possesses the largest gonad of the annual cycle (Figure 6.5; Tanaka, 1958). Subsequently, the gonad index declines in individuals as they enter the spent and resting stages (Figure 6.5; Tanaka, 1958). About 70–80% of the sea cucumbers collected in June–July are in the spent stage, while the remainder are either still in the mature stage or already in the resting stage (Figure 6.6; Tanaka, 1958). From August to October, the gonad is very small in size (70–100% of animals are in resting stage; Figure 6.6), and the gonad index is approximately 0.05% in both males and females. In November, the gonad has slightly recovered and the gonad index is 0.2% in both males and females (i.e., a majority of sea cucumbers are in the recovery stage). From December to March, the gonad index of both sexes increases from 0.3 to 0.7%, on average, which coincides with the beginning of gamete development and the growth stage. In May, the gonads develop more rapidly compared to earlier months; gonad index values of both males and females rise to 1.05% on average (Figure 6.5; Tanaka, 1958) and are considered mature (Figure 6.6; Tanaka, 1958).

In Mutsu Bay, North Japan, the breeding season of *A. japonicus* is from May to August based on several reports. From the end of May to the first 10 days of June, a large proportion of mature oocytes are found in the gonad (Yoshida et al., 2002). However, in Saga Prefecture (Southern Japan), the spawning season of *A. japonicus* lies within the March–May period (Figure 6.7; Ito and Kitamura, 1998). In April, the gonad index values and the oocyte diameter reach a peak, at >20% and 140 µm, respectively (Figure 6.8; Ito and Kitamura, 1998).

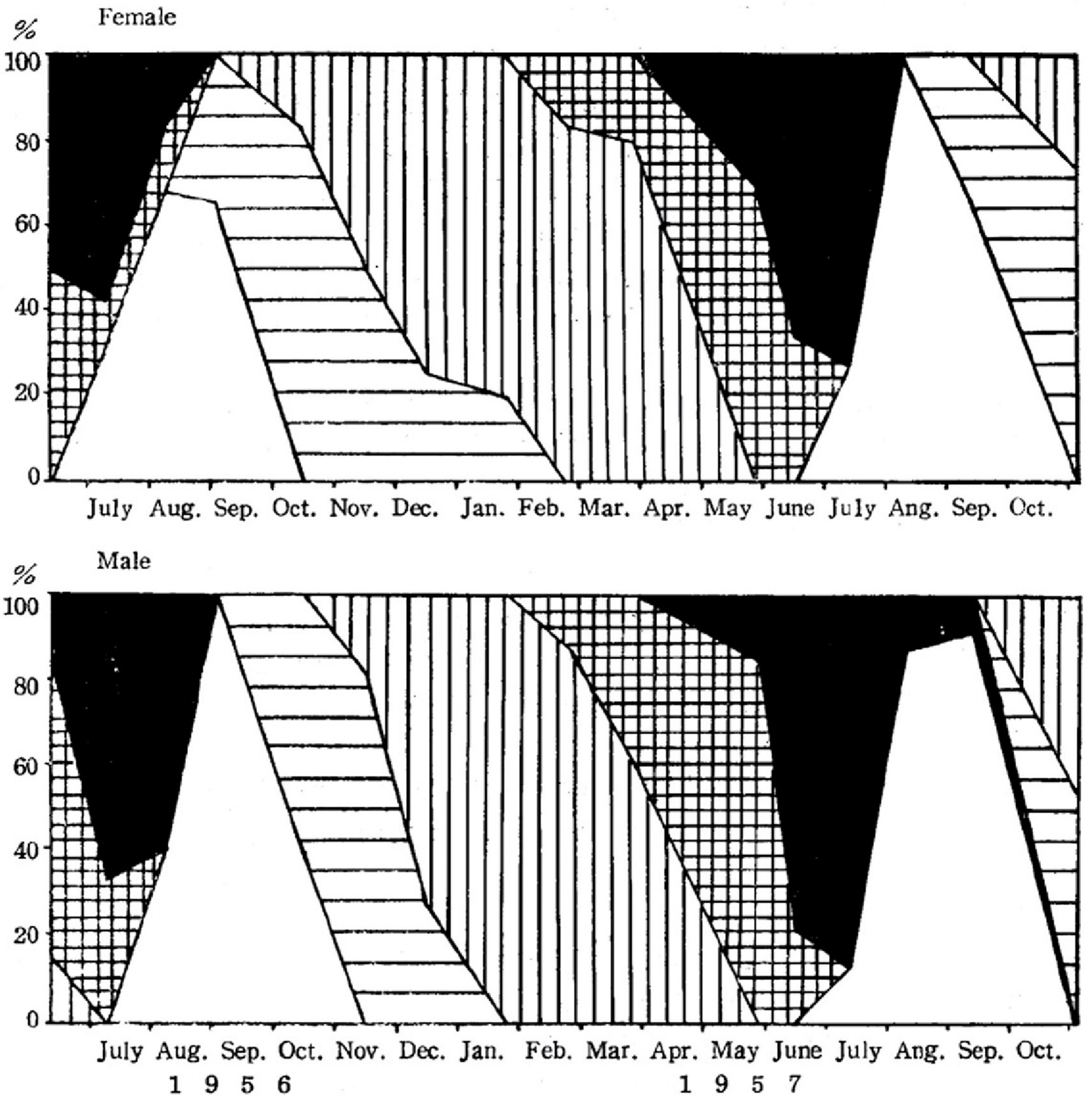

FIGURE 6.6

Reproductive cycle of *A. japonicus.* Reproductive cycles of females (upper panel) and males (lower panel) from June 1956 to November 1957, showing the proportions of different gametogenic stages. resting stage; early growth stage; growth stage; mature stage; spent stage.

From Tanaka, 1958

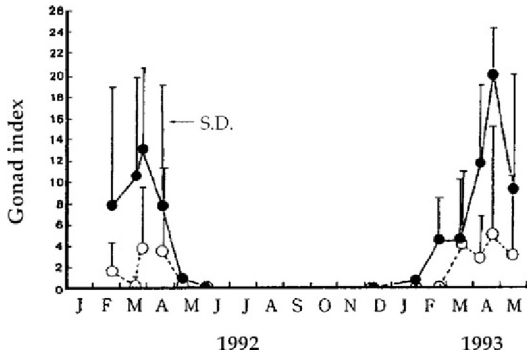

FIGURE 6.7

Seasonal changes in gonad index of *A. japonicus* in Kyushu Island, Southern Japan. Males, open circle/dashed line; females, solid circle/solid line. Vertical lines correspond to standard deviations.

From Ito and Kitamura, 1998

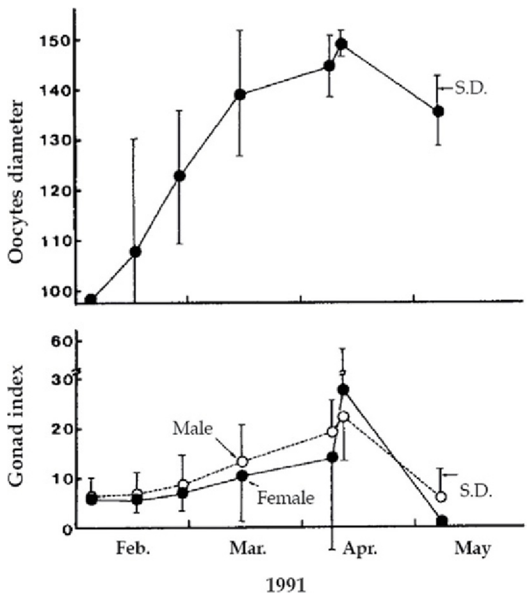

FIGURE 6.8

Changes in oocyte diameter and gonad index over time in *A. japonicus* in Kyushu Island, Southern Japan. Vertical lines correspond to standard deviations.

From Ito and Kitamura, 1998

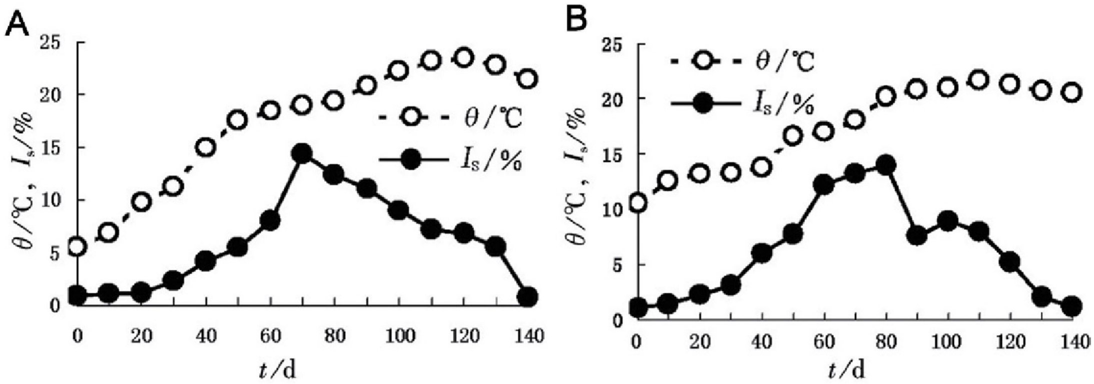

FIGURE 6.9

Relationship between the gonad index of *A. japonicus* and the sea surface temperature in the northern Yellow Sea (China). (A) Male gonad index between May and September 1991. (B) Male gonad index between May and September 1992. I_s, gonad index; t, time; d, day; θ, temperature.

From Zhou et al., 2001

6.3.2 CORRELATION WITH ENVIRONMENTAL FACTORS

In general, gametogenesis in holothuroids has been correlated with environmental factors, such as photoperiod and water temperature, while spawning is suggested to be triggered by changes in water temperature, food availability, light intensity, water turbulence, salinity, and phytoplankton blooms (Mercier and Hamel, 2009). However, few studies have explicitly tested the influence of these factors on the gametogenesis and spawning of *A. japonicus*.

What is known is that the gonad index of *A. japonicus* typically increases as the surface seawater temperature rises upward of 5 °C and peaks when seawater temperature reaches 20 °C (Figure 6.9; Zhou et al., 2001). The spawning season of *A. japonicus* coincides with seawater temperatures that fluctuate between 16 and 20 °C in the Nanao Bay of Japan (Tokuhisa, 1915) and in the northern Yellow Sea of China (Zhou et al., 2001). In the Peter the Great Bay, Sea of Japan (Russia), *A. japonicus* was determined to spawn when seawater temperature is between 18 and 25 °C (Gavrilova, 1995).

6.3.3 REPRODUCTIVE TRAITS

In a study conducted in Dalian, northern China, the sex ratio of *A. japonicus* appeared to be skewed toward females. Of the 110 mature specimens studied, 63 were females and 47 were males, yielding a sex ratio of 1.34:1 (Wang, 2009). In Japan, the sex ratio reported for *A. japonicus* is nearly 1:1 in central Honshu (Yanagisawa, 1998), although Sang (1963) reported it to be 1:1.09 in northern Honshu. Size at first sexual maturity is attained at the age of about two years, when individuals weigh ~250 g (wet weight) in China (although these values may vary; see Chapter 7 for details). During the gametogenically mature stage, there are 220,000–290,000 oocytes g^{-1} of ovary (Chen, 2003). The ripe

oocytes are uniformly elliptical in shape, about $160 \times 140\,\mu m$ in diameter (Tanaka, 1958). Fecundity in *A. japonicus* is related to body weight. Females are very fecund and can produce as many as 1–2 million oocytes; sometimes up to 10 million oocytes (realized fecundity) will be released in one single spawning event in China (Chen, 2003). In Japan, the number of oocytes per female was reported to reach 3 million (Yoshida et al., 2002).

6.4 CONTROL OF REPRODUCTION

6.4.1 OOCYTE MATURATION

Fully grown but noncompetent oocytes of *A. japonicus* possess a large nucleus, also called the germinal vesicle (GV), and are arrested at the first meiotic prophase. These oocytes can be triggered to complete final maturation (i.e., to reach competency) following hormonal or chemical treatments that trigger germinal vesicle breakdown (GVBD), thereby enabling the oocytes to advance to metaphase I or II and become fertilizable (Maruyama, 1985).

The prophase arrest of oocytes can be released in response to disulfide-reducing agents such as dithiothreitol (DTT) and 2,3-dimercapto-1-propanol (BAL) in *A. japonicus* (Maruyama, 1980). DTT acts on the oocyte surface to induce the production of a maturation promoting factor (MPF), which in turn triggers GVBD and the subsequent maturation process (Kishimoto and Kanatani, 1980; Kishimoto et al., 1982). The fully grown oocytes of *A. japonicus* usually have a cytoplasmic protuberance where the oocyte attaches to the follicle. Two premeiotic centrosomes are anchored to the protuberance by microtubules. After meiosis reinitiation induced by DTT, the GV migrates toward the protuberance. The GV breaks down after it migrates to the oocyte membrane on the protuberance side. The protuberance then contracts back into the oocyte and the first polar body extrudes from the site of the former protuberance (Pang et al., 2010).

It was also demonstrated that forskolin, theophylline, 3-isobutyl-1-methylxanthine and sodium nitroprusside inhibit the DTT-induced oocyte maturation in *A. japonicus*, which indicates that a decrease in the level of cAMP and cGMP is a necessary condition for the reinitiation of meiosis in the oocytes and that cyclic nucleotides are inhibitors of oocyte maturation (Karaseva and Khotimchenko, 1995). However, the activation mechanisms of these agents are still unclear and will need to be clarified.

6.4.2 REGULATION OF OOCYTE MATURATION

It has been shown that water extracts of radial nerves from five species of sea cucumbers, including *A. japonicus*, can induce oocyte maturation (Maruyama, 1985). Experiments on the maturation-inducing activity of radial nerve extracts indicated that they can cross-react effectively among the sea cucumbers examined (*Holothuria leucospilota*, *H. pervicax*, *H. moebi*, *H. pardalis*, and *Stichopus* = *Apostichopus japonicus*). The extracts act either on oocytes in isolated ovaries or on isolated oocytes with their follicle cells, but they are ineffective on oocytes deprived of follicle cells. The active mediators of oocyte maturation appear to be small peptides (Maruyama, 1985).

A gonad-stimulating substance-like molecule (GSSL), with a molecular weight of about 4.8 kDa, has been isolated from the radial nerve of *A. japonicus* (Katow et al., 2009). The activity of GSSL is similar to that of radial nerve extract, which induces GVBD at a concentration of $3\,mg\,mL^{-1}$ in 85% of oocytes. The amino acid sequence of GSSL was reported to be H_2N-VLSKQAHHHHHEGWSLP

GVPAEIDDLAGNIDYNIFKEQREKIK-COOH. The synthetic 43-amino acid GSSL generated from this sequence induced GVBD in 50% of oocytes at 6 μM. An N-terminal 21-amino acid peptide of the synthetic partial GSSL (GSSL-P1) induced GVBD in 80% of oocytes at 12 μM, indicating that GSSL-P1 is of sufficient length for GSSL activity. The GSSL peptide is located in granular cells in the hyponeural part of the radial nerve and in the epineural sinus beneath the radial nerve (Katow et al., 2009).

The gonad-stimulating substance-like peptide-containing polypeptide (GSSLP) has also been detected in the gonads shortly before the breeding season (May and June) and up to July (Ahmed et al., 2011). GSSLP shows considerable polymorphism among different organs, particularly in the gonads, associated with *N*-glycosylation and the formation of intramolecular disulfide bonds. In the ovary, GSSLP is expressed from March to June and corresponds to two bands at 113 and 100 kDa under reducing conditions. In July, only the larger band is weakly expressed. In the testis, GSSLP is detected first in April as two bands of 245 and 190 kDa under reducing conditions. The number of bands increases to five in June but decreases to three smeared bands in July. In the radial nerve and circumoral nerve ring, GSSLP corresponds to a single peptide of 170 kDa with little *N*-glycosylation and its expression level hardly changes throughout the year. In the ovary, GSSLP is detected in the follicle cells but not in the ooplasm. In the testis, the morula cells are localized close to the invaginated inner epithelium, but never in the male gametes. In July, gonad morula cells are rarely observed (Ahmed et al., 2011).

In addition, two small peptides (a pentapeptide and a heptapeptide) have been extracted and purified from tissues containing buccal ring nerve or longitudinal radial nerve of *A. japonicus* (Kato et al., 2009). The pentapeptide was identified as NGIWYamide (coined Cubifrin). It induces GVBD *in vitro* and ovulation of fully grown oocytes at a concentration less than 1 pM as well as spawning *in vivo* at 10 nM. The heptapeptide is less potent, inducing ovulation at 1 μM. In addition, a synthetic derivative of the pentapeptide, NGLWYamide, was 10–100 times more potent compared to the natural NGIWYamide (Kato et al., 2009).

6.4.3 REGULATION OF SPAWNING

The synthetic peptides NGIWYamide (Cubifrin-I) and NGLWYamide (Cubifrin-L) can also induce final oocyte maturation *in vitro* and a typical spawning behavior (Kato et al., 2009; Fujiwara et al., 2010b). The responsiveness of ovarian fragments containing oocytes >155 μm in diameter to Cubifrin-L *in vitro* is well correlated with the spawning success induced *in vivo* by Cubifrin-L injection. Mature sea cucumbers injected with Cubifrin-L display sequential reproductive behaviors, which comprise climbing up the side wall of the tank toward the water surface, waving of the head, and shedding of gametes. Gamete shedding starts about 60 and 80 min after the injection of Cubifrin-L in males and females, respectively, and is completed almost simultaneously in the two sexes about 2 h post-injection. Repeated injections of Cubifrin-L at intervals of about 10 days successfully induce multiple spawnings in males and females (Fujiwara et al., 2010b).

6.4.4 PROTEINS AND GENES INVOLVED IN GAMETOGENESIS

Yolk proteins are excellent biomarkers for understanding the process of ovarian development and its regulatory system. During oogenesis of oviparous animals, the growing oocytes accumulate large quantities of yolk materials, including proteins, lipids, carbohydrates, vitamins, and carotenoids, which supply essential nutrients to the offspring until feeding begins. The most abundant yolk protein in

many oviparous animals is vitellin, a precursor protein of vitellogenin (Fujiwara et al., 2010a). In sea urchin (Echinodermata: Echinoidea), the yolk protein precursor is generally termed major yolk protein (MYP), which is structurally distinct from vitellin/vitellogenin (Yokota et al., 2003). During oogenesis, the most abundant protein in the coelomic fluid of *A. japonicus* is also identified as MYP (Fujiwara et al., 2010a). The full-length cDNAs for two MYPs consist of 4600 and 4420 bp, with predicted protein lengths of 1365 and 1345 amino acid residues, respectively. The transcripts for both types of MYPs are detected in the respiratory tree, intestine, body wall, coelomocyte, testis, and ovary of *A. japonicus*. The transcript levels of both MYPs in the ovary are apparently elevated at late stages of ovarian development, whereas the MYP content of the ovary remains stable throughout ovarian development (Fujiwara et al., 2010a).

The *vasa* gene and protein are also helpful germline markers to study the origin and development of germ cells and gonads (Gustafson and Wessel, 2010). In *A. japonicus*, it has been found that the expression pattern of *vasa* coincides with the mRNA and protein levels during oogenesis (Yan et al., 2013). Intensive signals in oogonia decrease gradually with the development of oocytes. However, the pattern is different during spermatogenesis. The *vasa* mRNA level is the highest in spermatogonia, reduced in spermatocytes, low in spermatids, and absent in spermatozoa, but the *vasa* protein is restricted to spermatogonia and early spermatocytes. These expression characteristics of *vasa* persist in both male and female gonads throughout the reproductive cycle, indicating that *vasa* mRNA is a good marker for studying the origin and migration of germline cells in *A. japonicus*. In addition, the *vasa* protein is also vital in the development and differentiation of germ cells in *A. japonicus* (Yan et al., 2013).

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CHAPTER

BROODSTOCK CONDITIONING AND SPAWNING

7

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SUMMARY

This chapter focuses on the gametogenic maturation and spawning of *Apostichopus japonicus* and explores the influence of environmental factors on gamete release. It also presents and discusses artificial spawning induction techniques. Food supply and water temperature have been identified as important mediators of gonad maturation in *A. japonicus*. Temperature shocks, exposure to air under shade, and exposure to running water have been used to induce spawning under laboratory conditions for the purpose of sea cucumber seedling production. In recent years, scientists have also identified other factors, such as variations in salinity, pH and light, as potential inducers of gamete release in this species.

Keywords: *Apostichopus japonicus*; broodstock conditioning; environmental factors; maturation; sea cucumber; spawning behavior; spawning induction

7.1 COLLECTION AND CONDITIONING OF BROODSTOCK

7.1.1 GONAD DEVELOPMENT IN WILD POPULATIONS

Since the 1970s, Chinese aquaculture has relied mainly on the collection of sea cucumber broodstock from the field or breeding ponds (Sui et al., 1985). This practice has led to the development of knowledge on the reproductive cycle of *Apostichopus japonicus* (see Chapter 6 for a complete overview of gametogenic development). It was determined that sea cucumbers around the Shandong Peninsula are in gametogenic growth in November, although many individuals still possess gonads in the recovery phase. These late-maturing individuals will not initiate gonad growth until the following March or April. At that stage, it remains difficult to distinguish female from male gonads with the naked eye; instead, it requires microscopic examination. Around May, a visual identification of the sexes is possible by looking at the color of the more developed gonads. From the end of June to early July, the gonads are swollen and reach their maximum sizes in preparation for spawning (Chen et al., 1978; Wei and Wei, 2005).

Seawater temperature plays a crucial role in the reproduction of *A. japonicus*; it is always between 16 and 20 °C during the breeding season (also see Chapter 6). Along the coast of Shandong, Liaoning, Hebei, and the north of Jiangsu (China), where natural populations of *A. japonicus* occur, there

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Table 7.1 Spawning Periods of *A. japonicus* in the Wild

Locations	Spawning Periods	References
Hokkaido (Japan)	From late June to early September	Sui et al., 1985
Miyagi-ken (Japan)	From late June to early July	Sui et al., 1985
Onagawa (Japan)	From late July to late August	Sui et al., 1985
Dalian (China)	From early July to early September	Yu et al., 2005
Yantai (China)	From early May to late June	Yu et al., 2005
Beidaihe (China)	From mid-May to early July	Yu et al., 2005
Qingdao (China)	From late May to mid-July	Yu et al., 2005

is a shift in breeding period due to different seasonal patterns in seawater temperature (Table 7.1). Generally speaking, sea cucumbers living in southern latitudes of China spawn earlier than those in northern areas (Chang et al., 2006). For instance, sea cucumbers may breed from late May to mid-July in southern Shandong Peninsula (Qingdao and Rizhao City), whereas they begin to spawn from early June to late July in northern Shandong Peninsula (Yantai and Weihai cities) (Dong and Dong, 2009), and the breeding period in Liaotung Peninsula spreads from mid-July to mid-September (Zhou et al., 2001). In addition, even in the same area, interannual variations in spawning periodicity may occur (Table 7.1).

7.1.2 BROODSTOCK CONDITIONING

It is crucial to collect the broodstock when both male and female gonads are fully developed. Otherwise, immature gonads will typically stop developing when the animals are placed in captivity due to changes in environmental conditions or unsuited laboratory conditions, which could lead to gonad atrophy and interfere with spawning and gamete quality and/or quantity (Chang et al., 2006). Hence, the best time to collect the broodstock is no more than 5–10 days before the anticipated spawning period. Periodic examination of gonad samples is generally required to evaluate gonad development in prospective broodstock, whereby surgically extracted gonad tubules are examined under a microscope. However, experienced technicians can determine the level of maturity of the gonads from their texture and color (Figures 7.1A and 7.2A) (Liu et al., 2011). In recent years, well-established mariculture firms have been collecting broodstock only 1–3 days before spawning, keeping them in tanks without food until spawning (Liu et al., 2003; Wu, 2006). Water temperature in these holding tanks is controlled between 15 and 16 °C, the feces removed, and seawater changed every day until no traces of fecal matters are detected on the bottom of the tanks. The technicians then increase the water temperature progressively by two or three degrees.

When collected at such an advanced stage of gonad maturation, around 5–10% of these sea cucumbers may spawn upon arrival at the hatchery due to the stress of capture and transportation. When this happens, they should quickly be moved to other tanks, otherwise the released sperm can induce other individuals to spawn and have a negative effect on the mass production of zygotes. The remaining individuals will spawn after a few days in captivity under a controlled protocol (shown later).

The broodstock relaxed size (body length) is ideally greater than 20 cm and the total wet weight greater than 200 g, corresponding to an age of two years or more. Broodstock should exhibit

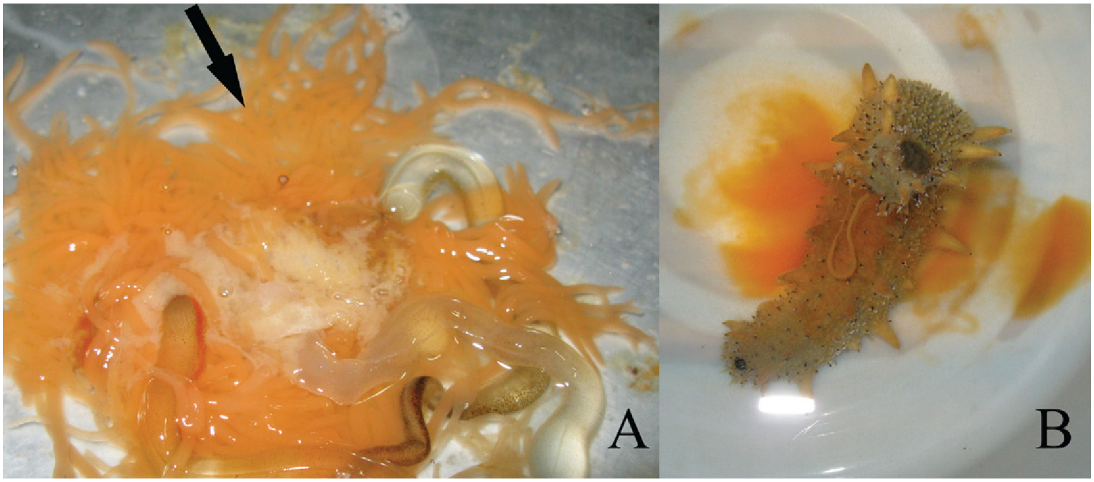

FIGURE 7.1

(A) Mature gonad of female *A. japonicus* (arrow points to gonad tubules). (B) Spawning of female *A. japonicus* (orange oocytes dispersing in the water column).

Photos by Shilin Liu

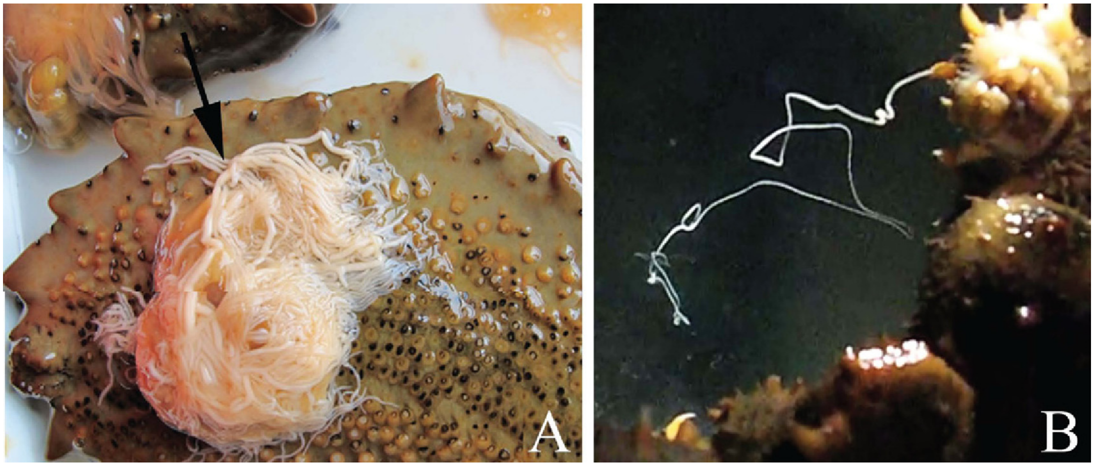

FIGURE 7.2

(A) Mature gonad of male *A. japonicus* (arrow points to gonad tubules). (B) Spawning of male *A. japonicus* (stream of white sperm dispersing in the water column).

Photos by Shilin Liu

a well-developed gonad and be free of any injury or discoloration on the body wall (Belova and Viktorovskaya, 2007; Yu et al., 2005).

Generally speaking, the environmental factors (such as pH, salinity and temperature) in nature are more stable than in ponds. Therefore, sea cucumbers from the wild always grow more quickly than those maintained under artificial conditions (in outdoor ponds or indoors), and their gonads always develop better and more synchronously; as a result, the fecundity of sea cucumbers and hatchability of their oocytes (i.e., number of auricularia/total number of fertilized oocytes) are both higher. Therefore, it is better to select individuals from the wild as broodstock. However, with the low numbers of wild *A. japonicus* remaining along the coast of China and the great demand for broodstock to sustain the needs of nurseries, adult broodstock grown in ponds are extensively used these days (Huang and Wang, 2007).

The aquaculture of *A. japonicus* has developed rapidly in China since 2000, with a corresponding increase in demand for juvenile sea cucumbers (seedlings with wet body weight from 2–20 g). Production of the seedlings typically requires a full year or more. In order to shorten this period for the sake of cost effectiveness, the majority of breeding farms have recently started to promote gametogenic maturation of broodstock in late March under controlled laboratory conditions (compared to May to June in the field). This manipulation, which brings broodstock individuals out of phase by about two months (Wang, 2009), requires rising seawater temperatures from 10–15 °C in winter at an earlier stage compared to conditions prevailing in nature.

More precisely, aquaculture farm technicians usually collect high quality broodstock (defined earlier) in mid-November when seawater temperature is between eight and 10 °C, and place these sea cucumbers indoors with adequate food. They are initially maintained at ambient temperature for three to five days in order to acclimate them to 10 °C. The seawater temperature is then increased to 13–15 °C at a rate of 0.5–1.0 °C per day. Finally, water temperature is increased to 15–16 °C for 7–10 days just before applying artificial spawning induction methods (presented in Section 7.3). These manipulations can shorten the time needed for adult sea cucumbers to reach full gonad maturity and entice them to spawn earlier than they would in the wild. The stocking density of broodstock is kept at <15 individuals m^{-2}, and the dissolved oxygen in water is maintained at 4–5 $mg\,L^{-1}$. During that period, half of the total water in each tank is changed every day by siphoning out the feces and excess food particles in an effort to keep a pollution-free environment. Feeding usually ceases at least one day before the anticipated spawning.

Feeding of broodstock (for animals grown in ponds) is an area that has not received any consensus so far; therefore, methods are inconsistent among different farms. As a general rule, the daily quantity of food provided should account for about 3–5% of the total weight of sea cucumbers (see Chapter 10 for more details). Research shows that a protein-rich diet can effectively benefit gonad development in other aquatic animals (Fernández-Palacios et al., 1997; Abdel-Fattah and Mamdouh, 2008; Rasanthi et al., 1996). Broodstock of *A. japonicus* should be fed a diet comprising 20–25% of protein. The major ingredients in the diet of adults maintained in ponds usually include powder of *Sargassum thunbergii*, *Sargassum polycystum*, *Laminaria japonica*, fragments of shrimp and scallop, and sea mud, with a handful of *Spirulina*, yeast, and egg yolk (Chen et al., 2012). The proportion of each ingredient varies across farms.

Two important parameters need to be measured to verify whether broodstock conditioning is progressing well. First, the gonad index needs to be increased to values above 10% (Chang et al., 2004). The following equation is used:

$$GI = GW / EW \times 100$$

where GI is the gonad index, GW the gonad wet weight, and EW the eviscerated wet weight. In addition, broodstock needs to be exposed to a certain cumulative heat, i.e., the integrated product of the number of degrees that seawater temperature rises above a given threshold value and the number of days in the rearing period during which this excess is maintained. It can be expressed as

$$K = N(T - C)$$

where K is the effective accumulated temperature, N the number of days cultivated, T the average temperature during culture, and C the biological zero point (i.e., the temperature above which gonad maturation occurs). The biological zero of *A. japonicus* is considered to be 7 °C and spawning becomes possible when broodstock has been exposed to an effective accumulated temperature of 800 °C (Sui, 2004).

7.2 SPAWNING BEHAVIOR

Sea cucumbers with mature gonads will release oocytes and spermatozoa (Figures 7.1B and 7.2B) almost immediately after artificial stimulation (such as desiccation, temperature shock, and running seawater; see Section 7.3). Prespawning behavior in *A. japonicus* involves moving to the corner of the tank, then moving up to the surface of the water and exhibiting sweeping head movements (Sui, 1990). This behavior indicates imminent spawning. The gonopore of *A. japonicus* is located on the anterior region, about 1–1.5 cm away from the mouth on the dorsal side of the body wall. Creamy white sperm is released from the gonopore and dispersed rapidly in the water column by the sweeping movements (Figure 7.2B). Sperm release will last for half an hour or more, resulting in the water becoming turbid. Sperm is a proximal signal for inducing females to spawn in *A. japonicus*, similar to what has been described in several other species of sea cucumbers (McEuen, 1988; Smiley et al., 1991; Mercier and Hamel, 2009). About 0.5–1 h after the beginning of male spawning, the female sea cucumbers will start to release orange oocytes in the water column (Figure 7.1B). The first and second cleavages occur after 80 and 100 min, respectively, in fast-developing propagules (Tan et al., 2012) (see Chapter 8 for details on embryonic and larval development). An average female sea cucumber may release between one and three million oocytes; the largest ones were found to release up to 6–10 million oocytes (Sui et al., 1985; Chen, 2003). Large ripe females may release oocytes one to three times in the same breeding period, although the quantity and quality of oocytes typically drops over time (Chen et al., 1978). Both indoors and outdoors, spawning usually occurs in the evening when light intensity is weakening, between 18:00 and 20:00, although exceptionally it may be triggered in daylight. *A. japonicus* exhibits negative phototaxis (Dong et al., 2011). When exposed to bright light during spawning, sea cucumbers will generally stop releasing gametes and will be contracting their body. They will resume spawning after the light has been turned off. For this reason, technicians usually keep spawning tanks in the dark (Liu et al., 2003).

Age at first sexual maturity is typically 2–3 years old; however, the relationship between body weight and sexual maturity is an important factor. Three-year-old sea cucumbers <100 g will not reproduce, whereas individuals under the age of two that have reached a wet weight >250 g (i.e., heavier than normal for their age) will always exhibit mature gonads (Yu et al., 2005). The oocytes released without artificial stimulation are generally of better quality than the oocytes obtained after artificial stimulation. Owing to the stable (or more natural) environmental factors, gonads of sea cucumbers

collected from the field grow more synchronously and more fully, reaching higher GI values. These high-quality oocytes will be released with only the slightest environmental stimulation. In contrast, sea cucumbers raised indoors have asynchronous development of gonad tubules, both intraindividually and interindividually. In this case, immature oocytes can be released along with mature ones after artificial stimulation. The rate of deformed oocytes and embryos may also be higher compared to individuals collected from the wild.

7.3 SPAWNING INDUCTION

Spontaneous spawning in *A. japonicus* may sometimes occur either upon or soon after collection (Yellow Sea Fisheries Research Institute, 1991). However, spontaneous spawning is neither logistically practical nor reliable enough for routine gamete collection. Several factors that mimic natural environmental cues have been demonstrated to affect spawning behavior and gamete release in *A. japonicus*, which may help regulate gamete supply. Spawning induction allows a better control of the spawning time, and may provide a greater number of fertilized oocytes at any given time (with a good ratio of male to female gametes), especially if applied strictly on mature individuals during the normal spawning period. This will benefit farms aiming to coordinate the development of large amounts of larvae or wishing to spread the effort across multiple batches.

7.3.1 TEMPERATURE SHOCKS

Thermal stimulation is the most common method of inducing spawning in many aquatic invertebrates (Loosanoff and Davis, 1963; Mercier and Hamel, 2009) including sea cucumbers (Mercier and Hamel, 2009, 2013). Temperature is also one of the key environmental drivers of gonad maturation and spawning in *A. japonicus* (Sui, 1990). Release of oocytes and spermatozoa in nature usually occurs when seawater temperature rises to 16–20 °C, in late spring or early summer (shown earlier). Therefore, rising seawater temperature may induce spawning in ripe sea cucumbers.

In order to obtain oocytes and spermatozoa during the natural breeding season (or out of phase under laboratory conditions), artificially increasing the seawater temperature by 2–3 °C is an effective method that can induce between 50–80% of sea cucumbers to spawn. However, greater temperature shocks, for instance an increase of 4–5 °C, may lead to developmental malformation and larval mortalities. Occasionally, sea cucumbers with full-grown gonads can spawn with a minimal thermal shock even when ambient temperature is still under 15 °C (presented earlier).

7.3.2 EXPOSITION TO AIR (DESICCATION)

In this method, the broodstock is gradually exposed to air and maintained in the dark by emptying the holding tank (Figure 7.3A). Dark conditions simulate evening light, i.e., the natural spawning time. After 0.5–1 h, these sea cucumbers are collected and moved to another tank filled with water at a temperature of about 18–20 °C (whereas the original temperature was between 15–17 °C). Soon thereafter (generally 1 or 2 h), *A. japonicus* will start climbing the walls of the tank to reach the surface of the water. The males will adopt the prespawning sweeping posture and start releasing gametes, soon followed by the females. This process may also be observed in nature, whereby sea cucumbers living in

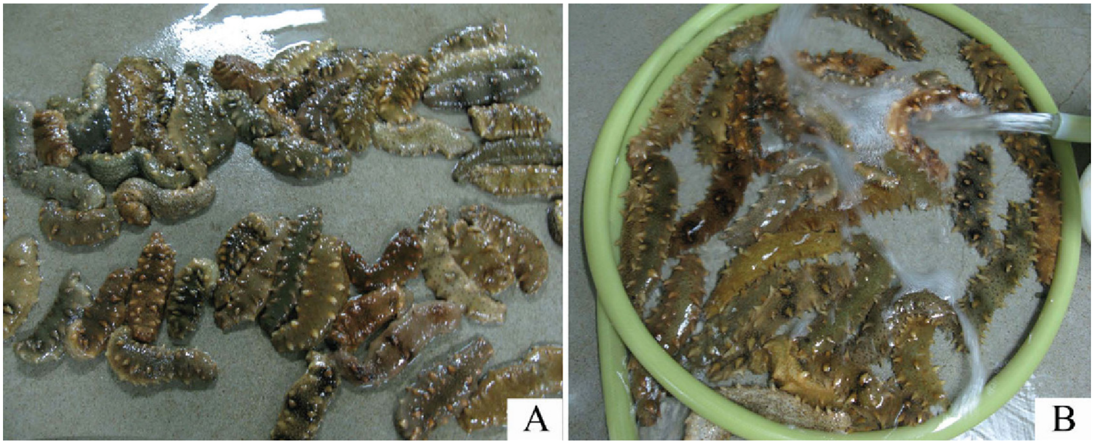

FIGURE 7.3

Techniques commonly used to induce spawning in *A. japonicus* include (A) desiccation and (B) running seawater.

Photos by Shilin Liu

the intertidal zone, exposed to air at low tide, will be stimulated to spawn (Sui et al., 1985). This method was also found to be a simple and efficient way to induce spawning in the tropical commercial sea cucumber *Stichopus horrens* (Hu et al., 2013).

7.3.3 RUNNING WATER

The movement generated by the flow of seawater can be another effective spawning trigger. Technicians stimulate spawning in broodstock using running seawater at a flow of about 0.5–1 $m\,s^{-1}$ for 20–40 min (Li et al., 2011; Zhang et al., 2010). It can also be combined with other above-mentioned methods to obtain optimal results (Sun et al., 1997). First, the sexually mature sea cucumbers are placed together in a container, then sprayed with water at ambient temperature or slightly higher by about one to two degrees (Figure 7.3B). Simultaneously, running water can also wash the body wall of the sea cucumbers and the containers in which the gametes will be released. Great care must be taken to adjust the flow so as not to inflict injuries to the body wall or trigger evisceration.

7.3.4 SALINITY AND pH VARIATION

Environmental factors thought to be involved in the control of holothuroid gametogenesis and spawning include temperature or temperature change, light intensity, photoperiod, salinity, tidal flux, food availability, and change in food type (Smiley et al., 1991; Mercier and Hamel, 2009). The normal salinity and pH of seawater in the natural habitat of *A. japonicus* is usually between 29 and 31 and 7.8–8.2, respectively. In practice, ripe sea cucumbers maintained in outdoor ponds can sometimes be

triggered to spawn by heavy rain falls, presumably due to the abrupt decrease in salinity and pH. Such environmental changes become a cue that can induce spawning under controlled laboratory conditions. In order to prevent spawning from being triggered at an inappropriate time, technicians will generally place ripe broodstock indoors when heavy rain is forecast.

7.4 ARTIFICIAL OOCYTE MATURATION

In ripe individuals of *A. japonicus*, the tubules of mature gonads measure 2–3 mm in diameter, and the gonad index is >10%. The female gonad is orange (Figure 7.1A) and the unfertilized oocytes oval-shaped under a microscope. The male gonad is milky white or yellowish (Figure 7.2A).

Oocytes extracted surgically from the gonad can not be fertilized because they have not undergone germinal vesicle breakdown (GVBD); they will waste away within 24 h. The intact germinal vesicle (~90 μm in diameter) includes a nucleolus measuring 15 μm in diameter. Cutting the gonad tubules into small pieces of about 1–1.5 cm and leaving them exposed to air in the dark for 2.5 h will stimulate GVBD; following this process, the oocytes will be round and will have completed their maturation, and fertilization rates will be >60% (Marine Biology Department of Shandong Marine College, 1977). Certain chemical agents (such as dithiothreitol/DTT, neuronal peptides, and Cubifrin) may also be used to induce oocyte maturation/competency and gamete release in *A. japonicus* (Pang, 2010; Shinichi et al., 2009; Atushi et al., 2010). Chapter 6 provides more details on those chemical mediators and the control of gamete maturation in *A. japonicus*.

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CHAPTER

8

DEVELOPMENT, SETTLEMENT, AND POST-SETTLEMENT GROWTH

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SUMMARY

The sea cucumber *Apostichopus japonicus* is an economically important species along the northwest Pacific Coast, including China, Democratic People's Republic of Korea, Republic of Korea, Russia, and Japan. In the present chapter, we summarize knowledge on the embryonic and larval development of *A. japonicus*, as well as the impacts of various environmental factors on these processes. *A. japonicus* is a dioecious broadcast spawner. Its life cycle can be divided into eight major stages: fertilization, blastula, gastrula, auricularia, doliolaria, pentactula, juvenile, and adult. The fertilized, mainly demersal eggs measure ~165 μm in diameter, and develop into freely rotating blastulae in 14 h at 20–21 °C. The embryos then develop into auricularia larvae over the following 34 h, at which time they gain the ability to feed. When the auriculariae grow to their maximum size of ~800–950 μm, they shrink and transform into doliolariae, and subsequently into pentactulae after completion of metamorphosis. The development of buccal and ambulacral podia indicates the onset of the juvenile stage. It takes almost two years for *A. japonicus* to reach sexual maturity, and the life span of this species is commonly estimated to be at least five years. The influence of environmental factors, such as temperature, salinity, and planktic food, on larval development and growth, as well as substrate selection during metamorphosis are discussed. Transitional functions of locomotory and feeding organs in larvae, behavioral characteristics during settlement, and interindividual variations in early growth rates are also examined.

Keywords: *Apostichopus japonicus*; development; embryo; interindividual variation; larva; salinity; sea cucumber; settlement; substrate selection; temperature

8.1 EMBRYONIC AND LARVAL DEVELOPMENT

Apostichopus japonicus is dioecious (exhibiting separate sexes) and is a broadcast spawner, i.e., the mature individuals shed gametes into seawater where fertilization occurs. The life cycle of *A. japonicus* can be divided into eight major developmental stages as follows: fertilization, blastula, gastrula, auricularia, doliolaria, pentactula, juvenile, and adult (Figure 8.1) (Liao, 1987; Wang et al., 2010; Hu et al., 2010).

The fertilized oocytes of *A. japonicus* are not exactly spherical, but ellipsoid to some extent. The longest and shortest diameters of the oocytes vary between 170 and 180 μm, and 160 and 175 μm,

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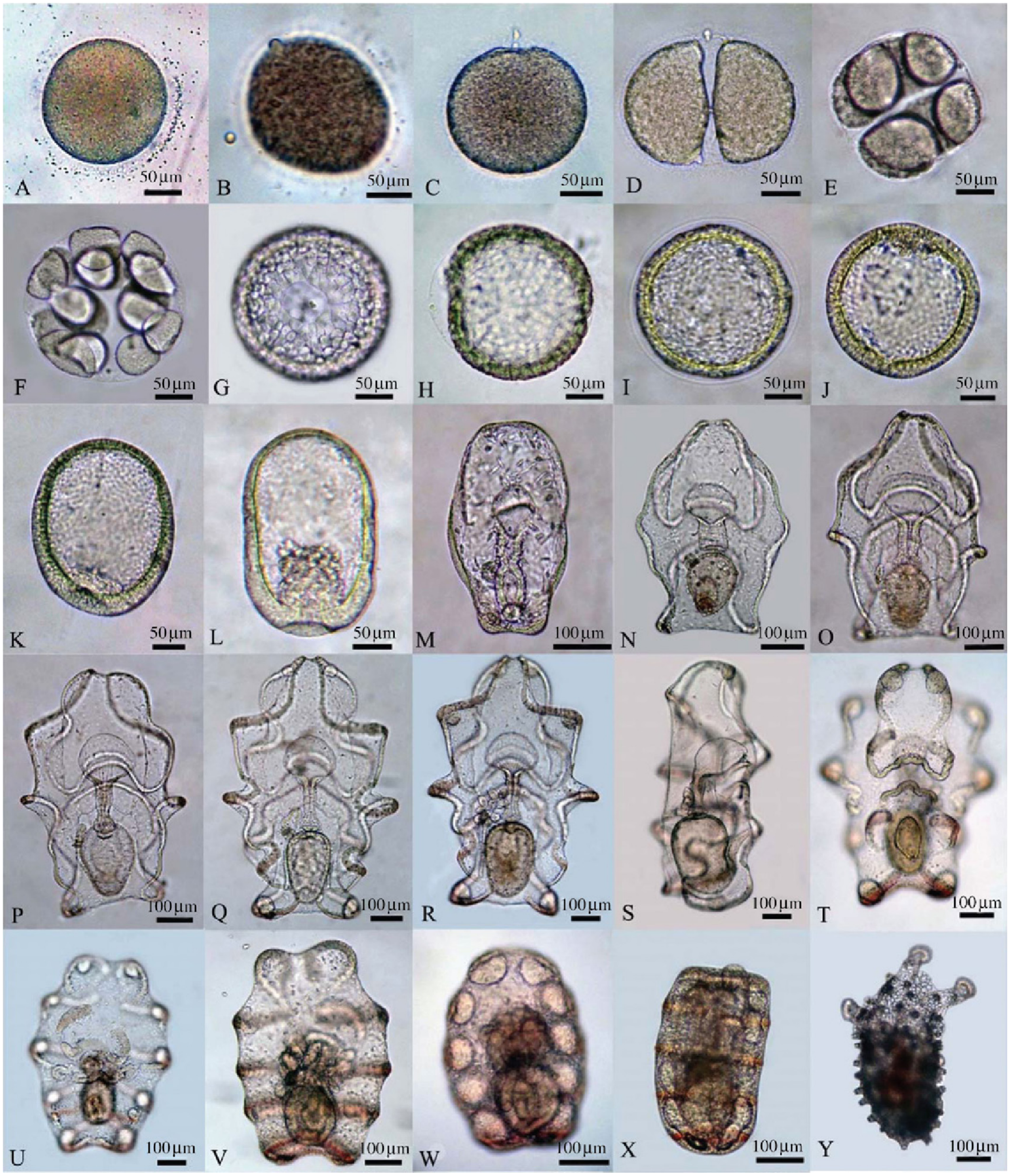

FIGURE 8.1

Development of *A. japonicus.* (A–B) Fertilized oocytes, (C) expulsion of polar bodies, (D) 2-cell stage, (E) 4–8-cell stage, (F) 16-cell stage, (G–H) blastula, (I) rotating blastula with expanded envelop, (J) late blastula, (K–M) developing gastrula, (N–O) early auricularia, (P–Q) mid-auricularia stage, (R–T) late auricularia stage, (U–W) doliolaria stage, (X) pentactula, and (Y) early juvenile with ambulacral podia.

From Qiu, 2013

respectively. Measurement of several batches of released oocytes indicate that the longer and shorter diameters exhibit a difference of 10 μm or less. Records of the size of fertilized oocytes (eggs) vary slightly; Zhang (1955) reported a diameter of 145–172 μm, whereas Liao (1997) and Zhu (2009) reported a diameter of 170–180 μm. Chen (1978) suggested that mature oocytes with a diameter <150 μm might be an indication of poor quality. However, the exact relationship between egg diameter and quality deserves further study.

After fertilization (time 0), the fertilization envelope elevates some 15–20 μm away from the egg itself (Figure 8.1A, B). The first and second polar bodies are expulsed about 20 and 40 min later, respectively (Figure 8.1C). The first hour is critical for polyploidy induction as the polar body expulsion and first cell division occur during this period. Ding et al. (2007) induced triploidy in *A. japonicus* by applying a hydrostatic pressure of 55 Mpa in the time interval between five and 55 min postfertilization, and tetraploidy between five and 61 min postfertilization at a pressure of 60 Mpa. Before the first cell division occurs, the polar bodies are observed on one side of the egg (Figure 8.1C, also visible in Figure 8.1D), with a visible fertilization envelope (Figure 8.1C). The two polar bodies remain visible until the embryo develops into the late blastula stage (Figure 8.1I) and the fertilization envelope breaks down. The late blastula starts rotating 11 h postfertilization, mostly in a clockwise direction (top view). Over the following 2–3 h, the blastula rotates increasingly faster, peaking at 0.3–0.5 revolutions per second, at which time the envelope expands and ruptures, then the ring-shaped ciliated late blastula starts to swim freely (Figure 8.1J). Over the next ~30 h, gastrulation occurs (Figure 8.1K–L), and the early auricularia develops.

Feeding starts at the early auricularia stage (Figure 8.1N), or about 48 h postfertilization. Although it was reported that the larvae of *A. japonicus* can survive for more than one week postfertilization without food, they never developed beyond the mid-auricularia stage when starved under laboratory conditions (Sun and Li, 2012a, b). The latter results also showed that starvation for six days postfertilization considerably reduced metamorphosis and survival rates compared to starvation for four days or no starvation (the latter conditions yielding similar results). To avoid mortality and obtain adequate growth and development, *A. japonicus* larvae should ideally be fed within 48 h of the opening of the digestive tract.

The mechanism underlying metamorphosis from auricularia to doliolaria and to pentactula is uncertain. However, recent research has shown that D1-like dopamine receptors are involved in larval metamorphosis of *A. japonicus* (Matsuura et al., 2009). Doliolaria larvae complete their metamorphosis to juveniles in 120 h when treated with 5–10 μM of dopamine and L-DOPA, and 50 μM of L-adrenaline and L-noradrenaline. Doliolaria larvae had to be exposed to dopamine or L-DOPA for at least 24 h. The D1-like dopamine receptor antagonists SKF87566 and LE300 (10 μM) inhibited metamorphosis by dopamine. However, the D2-like dopamine receptor antagonists sulpiride and nemonapride (10 μM) did not inhibit the effect of dopamine (Matsuura et al., 2009). In aquaculture production, technicians use plates covered by a biofilm to enhance metamorphosis rate rather than these chemicals (see Section 8.5 for details on substrate selection).

At a temperature of 20–21 °C, a salinity of 31, and a pH of 7.9–8.2, pentactula larvae can be observed for the first time to use their tentacles to attach to the substrate on the twelfth day postfertilization (Qiu, 2013). The following 3–5 days are the critical period for the pentactula to settle. Swimming speed of ~2.6 mm s^{-1} were measured in pentactulae, which is faster than any other stage, and it is considered to be a key character for substrate detection/selection and settlement. The just-settled larvae need 30–40 days, or even more due to marked individual size variations (see Section 8.7), to become a juvenile that is similar to the adult in appearance and organ functions (Qiu, 2013).

Table 8.1 summarizes the time line of major development stages and Figure 8.2 illustrates the typical growth pattern of *A. japonicus*.

Table 8.1 Time Table of Development in *A. japonicus* (0–48 h: 23–22 °C; 48 h to 28 d: 20–21 °C)

Time	Length (μm)	Stage and Characteristics
0 h	160–170	Fertilized oocyte (Figure 8.1A, B)
15–20 min	160–170	First polar body released (Figure 8.1C)
40–45 min	160–170	Second polar body released (Figure 8.1C)
1 h	190–200	1–2 cell stage (Figure 8.1D)
2 h	200–220	4–8 cell stage (Figure 8.1E)
3 h	200–230	16–32 cell stage (Figure 8.1F)
4 h	200–230	32–64 cell stage
7 h	200–230	Blastula stage (Figure. 8.1G–H)
11 h	210–230	Blastula-envelope expanded to a diameter of 240–260 μm; blastula start to rotate, but size remains stable (Figure 8.1I)
13–14 h	200–210/ 230–240	Hatching from fertilization envelope to become free-swimming (Figure 8.1J)
19 h	200–210/ 250–260	Late blastula hatching completed, transitional period between blastula and gastrula (Figure 8.1K)
21 h	180–190/ 270–290	Elongated gastrula (Figure 8.1L)
24 h	200–210/ 280–300	Gastrula stage, interstitial cell formed, deutostoma (mouth) site starts to appear
26 h	160–180/ 270–290	Gastrula stage, interstitial cell derived from the archenteron forms the hydrocoel primordium on the dorsal side
34 h	200–210/ 340–390	Late gastrula stage, dorsal pore and digestive tract formed; still nonfeeding (Figure 8.1M)
43 h	280–300/ 450–490	Early auricularia stage, calcareous ossicles in the posterior of the body appears
48 h	480–500	Digestive tract completed; able to feed (Figure 8.1N)
60 h	490–530	Hydrocoel formed on the dorsal side near junction between stomach and pharynx; star-formed calcareous ossicle formed, 20 μm in length
3 d	500–570	Left somatocoel derived from hydrocoel appears, still connected with hydrocoel
4 d	550–600	Left somatocoel and hydrocoel still joined (Figure 8.1 O)
5 d	650–680	Further development of left somatocoel and hydrocoel (Figure 8.1 P)
7 d	700–750	Hydrocoel expanded; right somatocoel visible
8 d	700–800	Left somatocoel separated from hydrocoel
9 d	800–850	Hydrocoel expanded, hyaline spheres appear (Figure 8.1 Q)
10 d	800–950	Five pairs of hyaline spheres, hydrocoel into finger-like branches, which will develop into circular canal and pentactula primordium; doliolaria larvae can be seen occasionally (Figure 8.1R–S)
11 d	800–380	Metamorphosis between auricularia and doliolaria (Figure 8.1T–W)

Table 8.1 Time Table of Development in *A. japonicus* (0–48 h: 23–22 °C; 48 h to 28 d: 20–21 °C) *(cont.)*

Time	Length (μm)	Stage and Characteristics
12 d	400–450	Early pentactula attaches using primary tentacles and swim with ciliary band; only 2–3 tentacles functional; critical period for finding substrate (Figure 8.1X)
13–15 d	390–430	Additional buccal podia develop, crucial stage for substrate selection; early attachment to substrate with buccal podia; sucker diameter ~50 μm
16 d	450–500	Settlement completed; first ambulacral podium present, with sucker diameter ~85 μm; tentacle sucker diameter ~60 um (Figure 8.1Y)
22–24 d	800–1000	Second ambulacral podium appears
27–28 d	1000–1200	First papillae on dorsal anterior side and third ambulacral podium appear

8.2 EFFECTS OF TEMPERATURE ON LARVAL DEVELOPMENT AND BIOLOGICAL ZERO

To explore the temperature effects on larval development, an experiment was carried out by Qiu (2013), where *A. japonicus* blastulae (6–7 h postfertilization) were cultured under various temperatures: 5, 10, 12, 15, 17, 19, 21, 25, 28, 31, and 34 °C. The growth curve and specific growth rate (SGR) in length are shown in Figures 8.3 and 8.4, respectively.

All embryos/larvae died within 24 h at 34 °C, and inside one week (before metamorphosis) at 31 °C. The optimum temperature for growth was 28 °C in the first three days; however, as time elapsed, growth rates decreased because of the death of the larger individuals (Figure 8.5). In the range between 15 and 25 °C, growth rates decreased as temperature dropped, whereas there was a clear decrease in survival rate after seven days between 21 and 25 °C, and 28 and 31 °C, while no clear differences occurred among treatments at 15, 17, 19, and 21 °C. In terms of growth rate, there was a significant difference between 15 and 17 °C, while no difference occurred among treatments at 17, 19, and 21 °C. In summary, the optimum temperature for larval rearing was found to be 21 °C, consistent with the results of Sui (1990), Liu (2008), and Li et al. (2011).

The embryos of *A. japonicus* only develop above a certain temperature. This temperature, which is also called developmental zero temperature, is calculated from the growth curve, using the equation

$$(T_1 - T_0)t_1 = (T_2 - T_0)t_2$$

then

$$T_0 = (T_1 t_1 - T_2 t_2) / (t_1 - t_2)$$

In the equations, T_0 represents developmental zero temperature, T_1 and T_2 represent rearing temperature in the experiment, and t_1 and t_2 represent the developing time (hour) from blastula stage to a certain larval length. Twelve applicable sites on the curve were selected to calculate the developmental zero temperature. The results of Qiu (2013) indicated that the developmental zero temperature for *A. japonicus* is 8.87 ± 0.63 °C (mean ± S.D.). This threshold was verified by the fact that embryos/larvae cultured

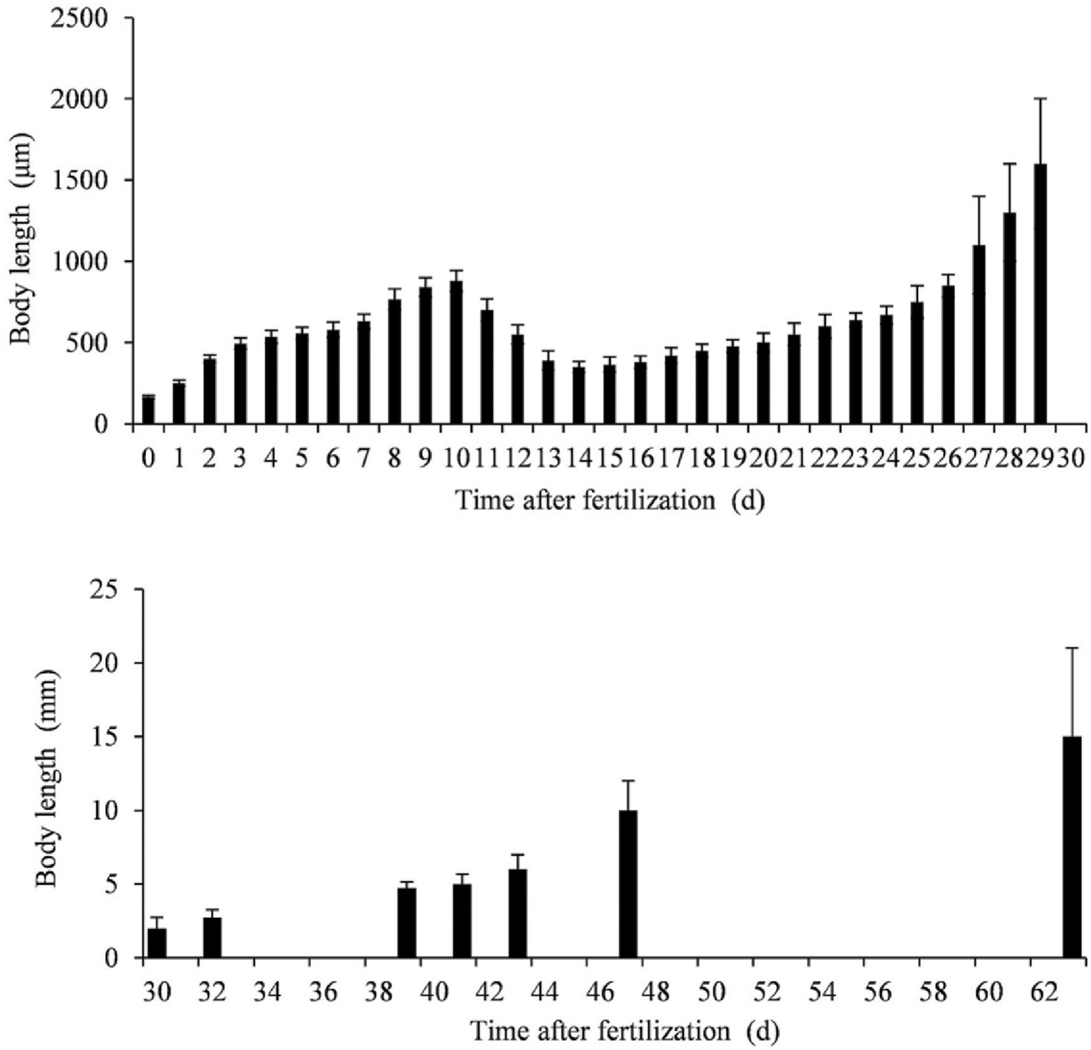

FIGURE 8.2

Growth of *A. japonicus.* Body length increment (mean ± S.D.) of larvae (top panel) and juveniles (bottom panel).

From Qiu, 2013

at 10 °C could still develop, although development was much slower (Figure 8.6). In contrast, embryos cultured at 5 °C stopped developing, and most blastulae shrunk from 200 μm to <160 μm after two weeks, while the others broke down (Figure 8.7). Overall, doliolaria larvae were obtained in 11 days at 20–21 °C under experimental conditions, close to the value of 8.5 days at 24 °C determined from the biological zero model. Li et al. (2011) demonstrated that 35% of larvae developed into doliolariae on the eighth day at 24 °C, which confirmed the results.

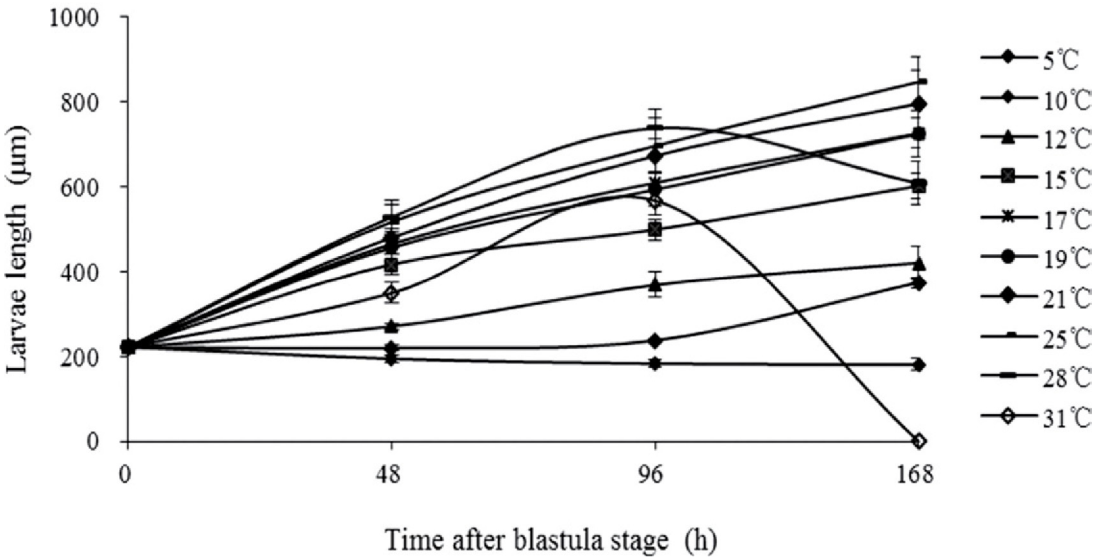

FIGURE 8.3

Influence of temperature on growth in *A. japonicus*. Length increment (mean ± S.D.) in larvae reared at various temperatures.

From Qiu, 2013

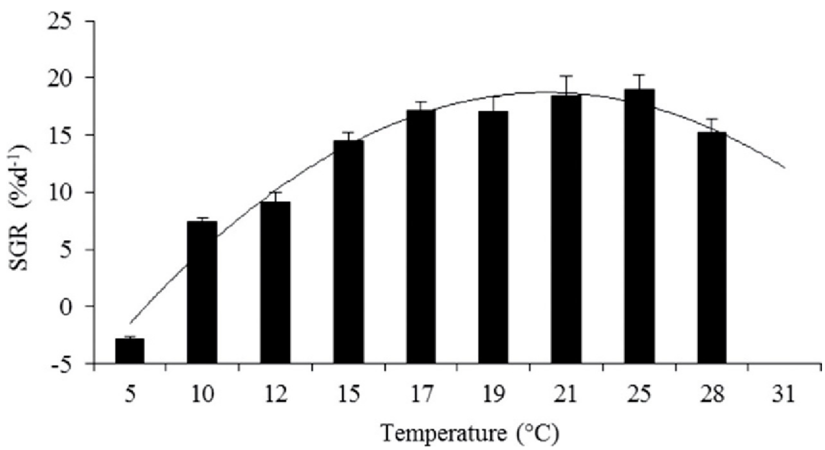

FIGURE 8.4

Larval specific growth rate (SGR) of *A. japonicus* during the first week of development at different temperatures. SGR ($\% \, d^{-1}$) = $100 \times (\ln L_2 - \ln L_1)/T$, where L_1 represents initial larval length, L_2 represents larval length on the seventh day, T represents 7 (days). Data shown as mean ± S.D.

From Qiu, 2013

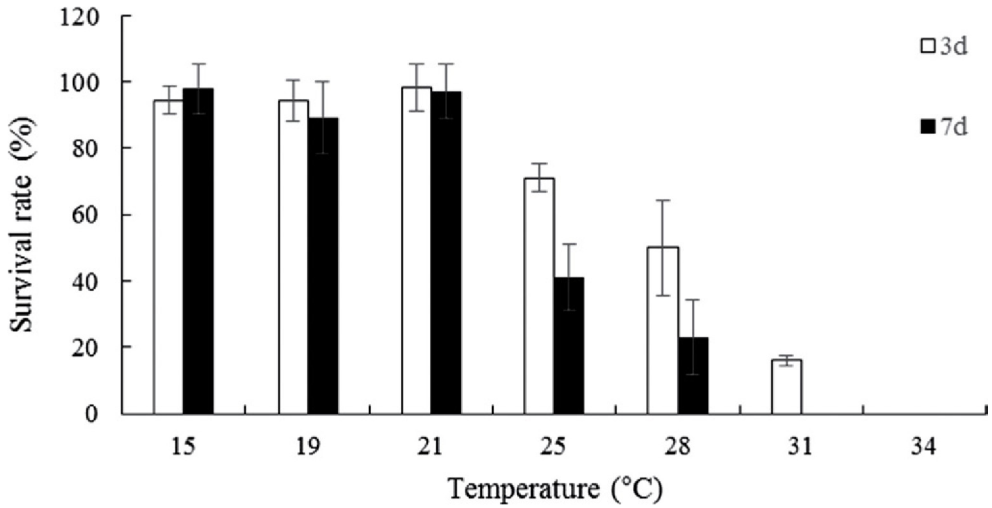

FIGURE 8.5

Survival rate of *A. japonicus* larvae (mean ± S.D.) after three and seven days at different culture temperatures.

From Qiu, 2013

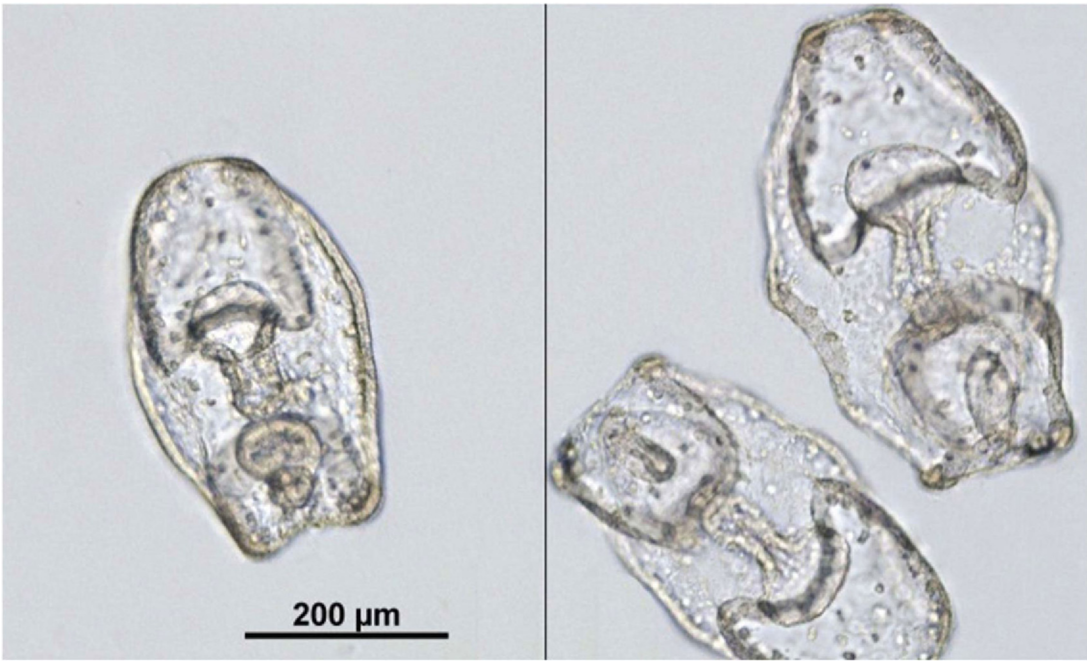

FIGURE 8.6

Larvae of *A. japonicus* seven days postfertilization (left panel) and 14 days postfertilization (right panel) during culture at 10 °C.

From Qiu, 2013

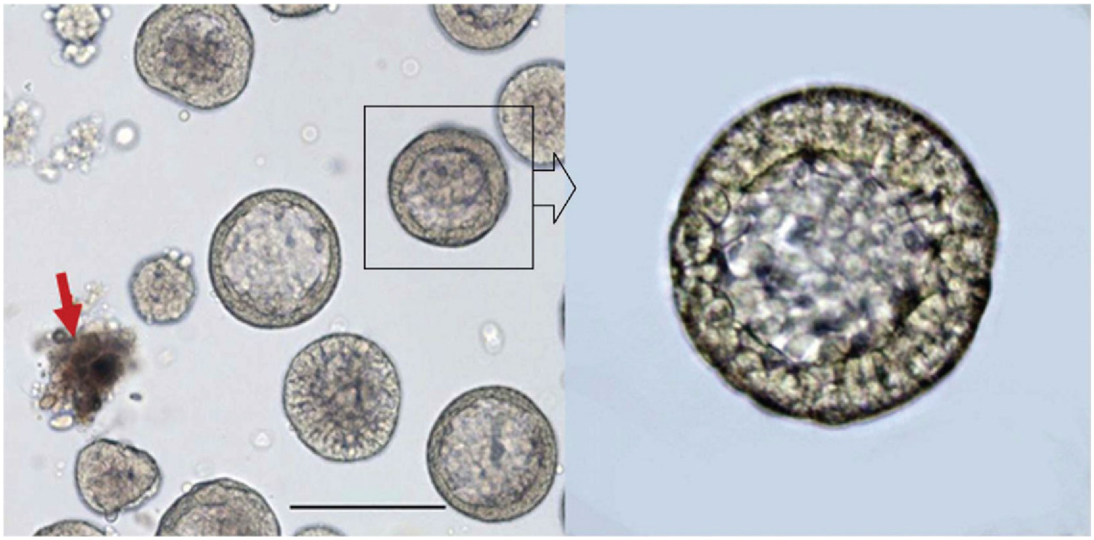

FIGURE 8.7

The shrunken and deformed embryos of *A. japonicus* cultured at 5 °C for two weeks. Arrow shows a disintegrating embryo; right panel shows a close-up of shrunken embryo. Bar on left panel = 200 μm.

From Qiu, 2013

8.3 EFFECTS OF SALINITY VARIATIONS ON GROWTH, DEVELOPMENT, AND VITALITY OF EMBRYOS AND LARVAE

Heavy rainfall is known to affect coastal water salinity, suggesting that organisms that reside in these environments must be able to tolerate important fluctuations. This prompted investigations into the effects of salinity on the growth, development, and locomotory speed of larvae of the sea cucumber *A. japonicus* under laboratory conditions. Results from Qiu (2013) indicated that early auricularia larvae cultured at a salinity of 31 moved the fastest ($0.64 \pm 0.06\,mm\,s^{-1}$; mean ± S.D.), followed by those reared at 35 ($0.57 \pm 0.07\,mm\,s^{-1}$), 26 ($0.55 \pm 0.06\,mm\,s^{-1}$), 23 ($0.54 \pm 0.06\,mm\,s^{-1}$), and 38 ($0.43 \pm 0.08\,mm\,s^{-1}$) (Figure 8.8). Over the seven-day period of cultivation, larvae grew fastest at a salinity of 31, followed by 26, 23, 35, 20, and 38, which indicates that the optimum salinity for larval rearing is approximately 31 (Figure 8.9).

Analysis of larval locomotory speed and development showed that speed was correlated with larval length on days 3, 5, and 7 ($r = 0.902/0.893/0.878$ and $P = 0.002/0.003/0.004$, respectively). However, when salinity was decreased to 20 or increased to 40, locomotory speed was significantly reduced. No larvae completed metamorphosis at salinity levels of 18, 20, and 40, whereas few developed into juveniles at levels of 23 and 38, which identify these values as salinity thresholds for metamorphosis and indicates that a salinity between 26 and 35 is suitable, with an optimum around 31 (Figure 8.10).

Kashenko (2000) reported that embryos of *A. japonicus* (blastula to gastrula stages) experiencing low-salinity acclimation for 18 h developed enhanced tolerance to low salinity, down to values of 20–22. Larvae acclimatized to a salinity of 22 developed normally, while among those acclimatized to

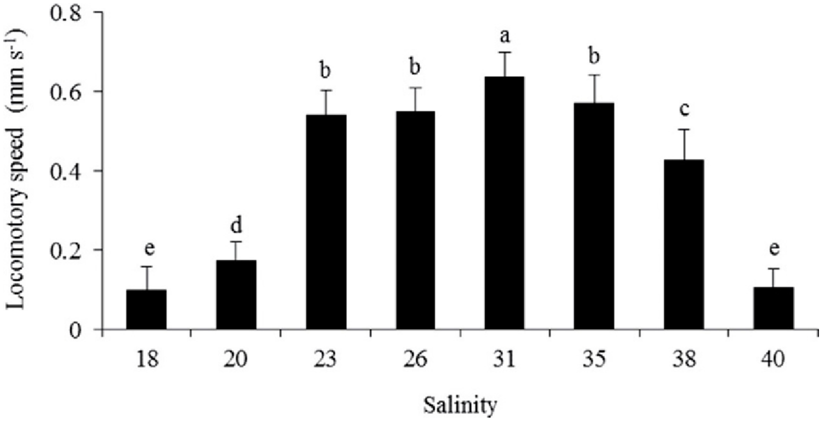

FIGURE 8.8

Locomotory speed measured in early auriculariae of *A. japonicus* on the third day of development at different salinity levels. Means (±S.D.) with different letters indicate significant differences ($P < 0.05$, one-way ANOVA).

From Qiu, 2013

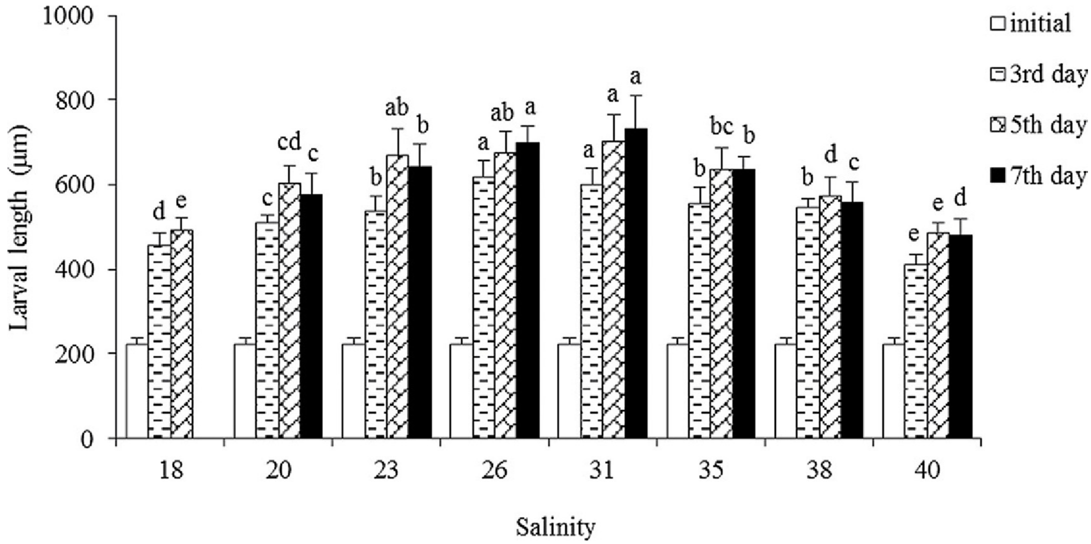

FIGURE 8.9

Mean larval length (±S.D.) of *A. japonicus* reared at different salinity levels after three, five and seven days. Different letters represent significant differences among salinity levels ($P < 0.05$, one-way ANOVA).

From Qiu, 2013

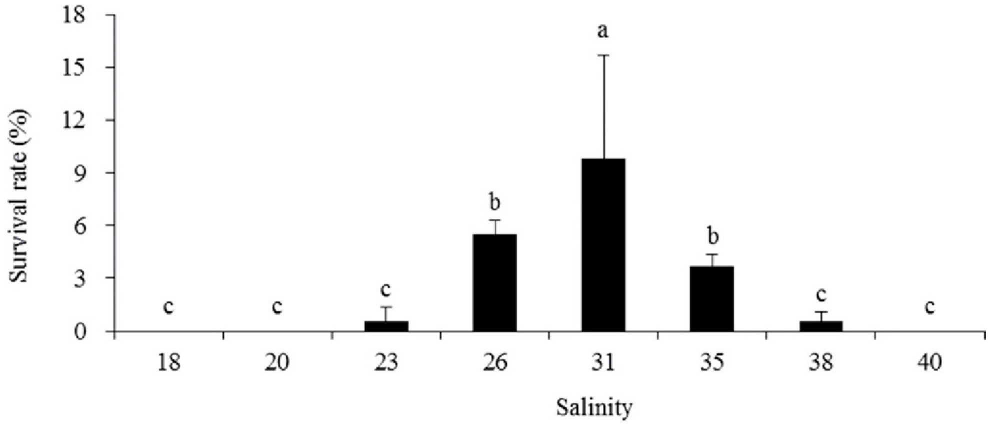

FIGURE 8.10

Survival rate of juveniles of *A. japonicus* after one month at different salinity levels. Initial abundance was ~3200 eggs in each 8 litre tank. Means (±S.D.) with different letters are significantly different ($P < 0.05$, one-way ANOVA).

From Qiu, 2013

20 only a small proportion developed normally. Li et al. (2011) reported that *A. japonicus* larvae could not live at a salinity of 15 (survival < 24 h) or 40 (survival < 9 d), and that growth rates were reduced at salinities of 20 and 40. Wang et al. (2009) demonstrated that the larvae died within 12 and 36 h when they were reared at salinities of 13 and 17, respectively. In some experiments, the larvae reared at a salinity of 20 or less could not complete metamorphosis (Qiu, 2013). However, low salinity (below the minimum tolerance limit of 22) only had an irreversible effect after exposures of a certain duration. Larvae that experienced a salinity of 18 for less than 6 h showed enhanced locomotory ability, and their growth and development completely recovered when salinity was returned to normal (31) inside that time frame, while those treated for more than 24 h were severely damaged and could not recover (Figure 8.11).

Larvae reared at a salinity of 18 were distended and stopped developing at the early auricularia stage by the third day of culture; in addition, the hydrocoel primordium formed without further development in the following days until death (Qiu, 2013; Figure 8.12A). Larvae at a salinity of 20 formed the hydrocoel and left somatocoel by the fifth day; however, further development was not observed over the following days, and their shape was also distended. In those larvae reared at salinities above 23, the left and right somatocoels developed normally. Results from larvae reared at 40 were similar to those reared at 18, with no further development of the hydrocoel primordium after its generation, and all the larvae dying before metamorphosis. However, the contracted shape of these larvae differed from that of larvae reared at 18. In contrast, at a salinity of 38, the development of the hydrocoel primordium was not inhibited and >50% of the larvae developed the hydrocoel normally (Qiu, 2013).

Tolerance to low salinity in *A. japonicus* offspring varies ontogenetically. The middle auricularia, which has a fully developed hydrocoel and somatocoel, is more sensitive than other stages, including blastula, gastrula, early auricularia, and late auricularia (with hyaline spheres). Although blastulae were

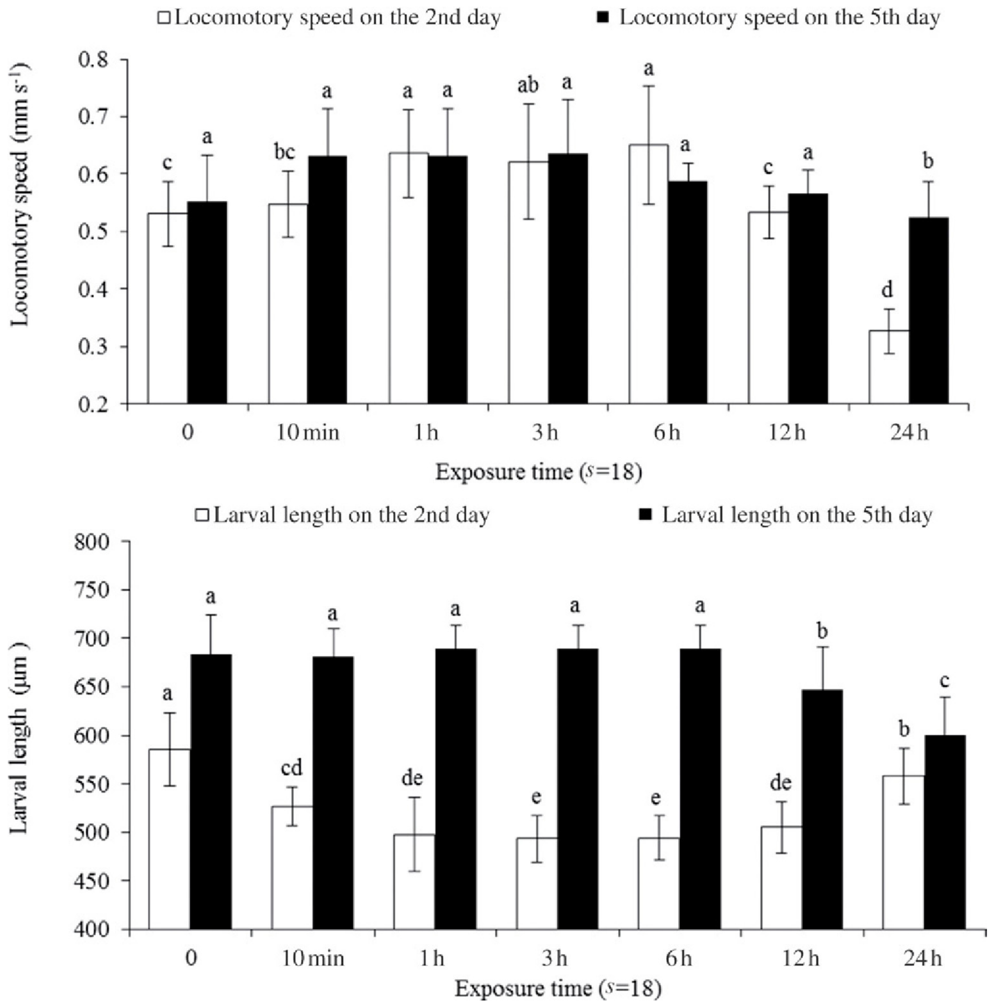

FIGURE 8.11

Locomotory speed of *A. japonicus* larvae. Larval speed (top panel) and length (bottom panel) on the second and fifth day after exposure to a salinity of 18 over time (0–24 h). Means (±S.D.) with different letters are significantly different ($P < 0.05$, one-way ANOVA).

From Qiu, 2013

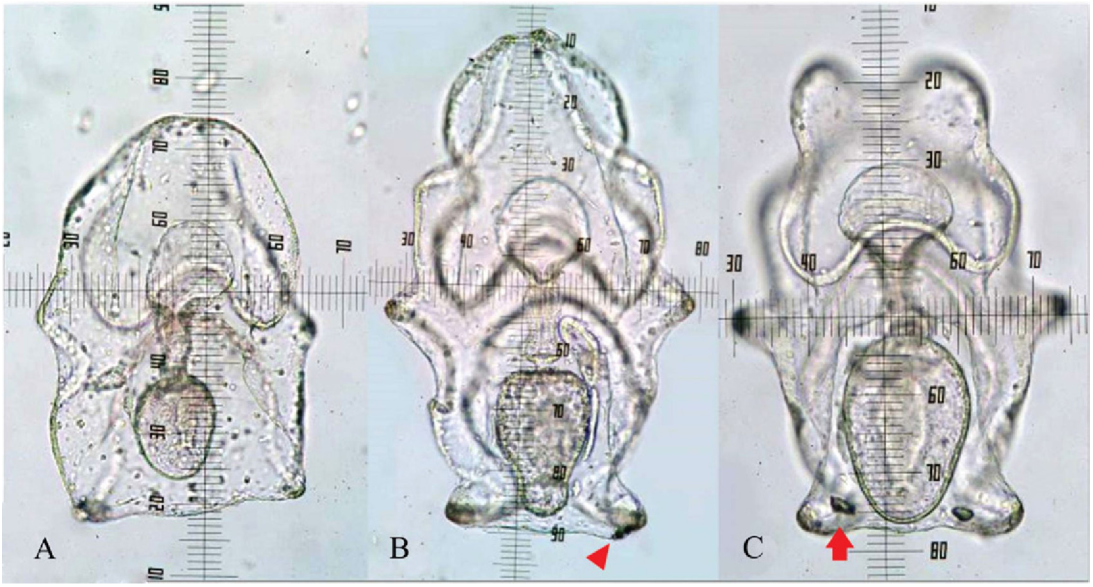

FIGURE 8.12

Typical larval development features of *A. japonicus* at different salinity levels on day 7. (A) Salinity = 18, dying larva was distended, development stopped at the early auricularia stage. (B) Salinity = 31, larva with normally developed hydrocoel and somatocoel, arrowhead showing normal calcareous ossicle. (C) Salinity = 40, larva was contracted, with two large calcareous ossicles (arrow). Minor grid represents 10 µm.

From Qiu, 2013

not able to swim out of the low salinity horizon (salinity between 20.0 and 20.5), they survived in it. The fertilization envelope around the early blastula may therefore act as a protective film. Larvae that develop to gastrula, early and late auricularia, and doliolaria can initially migrate out of this low salinity horizon, presumably avoiding the adverse effects of reduced salinity in surface layers of seawater (Kashenko, 2002).

Under natural salinity conditions, the larvae of *A. japonicus* usually develop one calcareous ossicle in the left posterior side of their body, which extends out during the early auricularia stage (Qiu, 2013). The ossicle is usually spindle-shaped and its average size is 42 ± 5.4 µm at the late auricularia stage (Figure 8.12B). When the first ambulacral podium is generated, this ossicle grows to 50–60 µm in length, with a variable shape. By the seventh day, the ossicles were 27.7 ± 9.5 µm in the dead larvae that had been reared at a salinity of 18, whereas in larvae reared at 40, the ossicles had reached 43.7 ± 3.6 µm. Although the length of ossicles was similar to those reared under normal salinity conditions, their three-dimensional size was larger. Furthermore, 40% of the surviving larvae reared at a salinity of 40 had a pair of calcareous ossicles (Figure 8.12C), whereas this was the case in only 10% of surviving larvae reared at 18 (Qiu, 2013).

8.4 EFFECT OF SUSPENDED SEDIMENT ON LARVAL SURVIVAL AND SETTLEMENT

Silt and microbial detritus make an important component of coastal surface sediment in the coastal areas of China; when resuspended, this matter is responsible for a large part of seawater turbidity. Suspended sediment is also known as suspended solid, or total suspended solid (TSS). Its content is not stable, but fluctuates within a certain range due to ocean currents and wind. Suspended sediment affects fish respiratory systems and may ultimately inhibit their growth and survival. For instance, Sutherland and Meyer (2007) found that a concentration of suspended sediment of more than 100 mg L^{-1} had an influence on the gills of the minnow, and that 500 mg Ll^{-1} of suspended sediment would seriously damage them. Other marine organisms may be influenced. Xing et al. (2012) demonstrated that different turbidity levels inhibited the photosynthetic efficiency of zooxanthellae associates with corals to different degrees, and also affected their density. Greater inhibition occurred with increasing turbidity. Therefore, suspended sediment in natural seawater may significantly impact the organisms living in it, either directly by affecting normal biological growth and metabolism, or indirectly by changing the transmittance of the water and other environmental factors.

To determine the effects of TSS on the larvae of *A. japonicus*, seabed surface sediment collected from Yuehu lagoon (China) was used to simulate suspended sediment in the laboratory (Qiu, 2013). Mean organic matter content was 5.4 and 4.0% in fresh and boiled sediment, respectively. TSS concentrations of 50–550 mg L^{-1} were tested to determine their effects on the survival and settlement rate (number of settled juveniles/number of fertilized oocytes). Settlement under different levels of TSS did not differ from the control, except at 50 mg L^{-1} of fresh sediment and 150 mg L^{-1} of boiled sediment. Settlement rates of larvae cultured at TSS concentrations of 150–300 mg L^{-1} were higher, but not significantly different from the control treatment (Figure 8.13).

Suspended sediment in natural seawater (usually < 150 mg L^{-1}) may not always cause lethal damage to sea cucumber larvae, thus it might not be the main reason why larval survival rate is low in the wild. On the contrary, suspended sediment that is rich in organic detritus and microbes may provide additional food for the larvae, and be favorable (to a certain degree) for their growth and settlement.

8.5 SUBSTRATE SELECTION

Hard smooth substrata are required for the pentactula larvae of *A. japonicus* to settle. The structure of the buccal cavity and the first ambulacral podia diameter (~50–85 μm, Table 8.1) determine the fine-scale substrate selection process. As a result, the number of juvenile *A. japonicus* settled on fine nylon monofilament was shown to be significantly less than that on polythene plastic sheets with a smooth surface (Li et al., 2010).

Monitoring newly settled juveniles showed that those with only one ambulacral podium had minimal migration ability. The first ambulacral podium acts as an anchor, thus juvenile sea cucumbers could only feed on the resources around them for a short period of time, generating numerous bare spots on the conditioned plastic-sheet substrate (Qiu, 2013). Based on this behavioral trait, the biofilm available on the substrate emerges as a key element for newly settled juveniles to survive. Du and Yu (2010) found that as the biofilm biomass on the substrate decreased, more settled juveniles died of starvation. Therefore, developing a proper food source (biofilm) on the substrate before larval settlement is necessary for successful production of seedlings (juveniles).

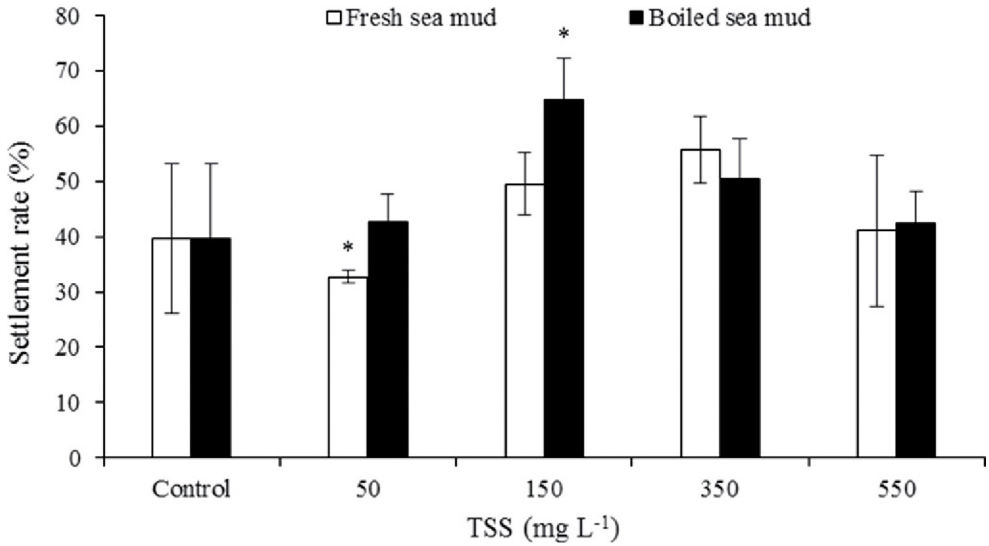

FIGURE 8.13

Settlement rate of *A. japonicus* larvae (mean ± S.D.) in different concentrations of total suspended solid (TSS). Asterisks (*) indicate significant differences between levels within a treatment (one-way ANOVA, $P < 0.05$).

From Qiu, 2013

In addition, the type of biofilm is also an important factor that influences larval settlement. Li et al. (2010) studied the settlement rate of *A. japonicus* larvae on different biofilms developed on plastic sheets. Results showed that the highest settlement rate occurred on diatom-based films, followed by effective microorganisms (EMs)-based films (for more details on EM, refer to Daly and Stewart, 1999) and natural microbial films; the plastic sheets without biofilm had the lowest settlement rate. It was also found that the plastic sheets coated with tissue extracts of adult *A. japonicus* appeared to favor larval settlement compared to the control without adult extract (Li et al., 2010). Sun (2012a, b) studied the influence of benthic diatoms on larval settlement and found that the presence of certain species of diatoms (e.g., *Nitzschia* sp.) could significantly enhance metamorphosis and settlement rates. Apart from the biofilm, the location/orientation of substrata can influence settlement rates according to Du et al. (2010), who indicated that horizontal plastic sheets yielded better settlement rates than vertical sheets (Du and Yu, 2010).

In summary, the substrate material and structure, the type of biofilm, and even the orientation of substrata can influence larval settlement in *A. japonicus*.

8.6 BEHAVIORAL CHARACTERISTICS DURING SETTLEMENT AND SHIFT IN FUNCTION OF LOCOMOTORY AND FEEDING ORGANS

The morphological structure of various organs underlies their function and affects the behavior of an organism. The ciliary rings of the auricularia larva in sea cucumber are the basis of their planktonic and suspension-feeding life. The ciliary rings and body contractions also enable the early pentactula

larva to move efficiently in the water column. When the cilia of the pentactula disappear, the larva fully metamorphoses into a benthic life stage called juvenile. One of the key characteristics of completion of metamorphosis is the formation of ossicles. Both the locomotory and feeding organ development and differentiation relate to specific ossicle types.

The first ossicle to appear in the body wall of the pentactula is X-shaped (Figure 8.14A). The X-shaped ossicle continues to develop into a sieve-like structure with a pyramidal central area, which is known as T-shaped or table-shaped ossicle (Figure 8.14C). While the function of ossicles in the body wall remains unclear, they are presumed to have existed for hundreds of millions of years according to fossil records (Wang and Chen, 1992; Huang et al., 2013). The calcareous ring found in the adult is the largest skeletal structure in the sea cucumber; this calcareous ring can be traced back to the 16-day-old juvenile (Figure 8.14B), when it is formed by the aggregation of ossicles bearing resemblance to those found in the body wall.

Before the ambulacral podia develop, the pentactula feeds and moves with the buccal podia (or buccal tentacles) only (Figure 8.15). The buccal podia/tentacles (Figure 8.15D, E) have C-shaped ossicles surrounding their stalk, and their lumen is full of fluid. The buccal podium is flexible and can bend, stretch out, and retract with the help of the hydraulic system and supporting ossicles. The locomotory pattern changes when the ambulacral podia develop. The ossicles in the ambulacral podia are different from those in the buccal tentacles; i.e., they are not C-shaped, but instead T-shaped ossicles; furthermore, short irregular ossicles surround the stalk and madreporic plate in the terminal nodule of the podia (Figure 8.15A–C). The first ambulacral podia with discontinuous T-shaped ossicles are not flexible; they cannot easily stretch out and draw back like the buccal podia/tentacles. Therefore, late pentactulae with only one ambulacral podium have limited mobility.

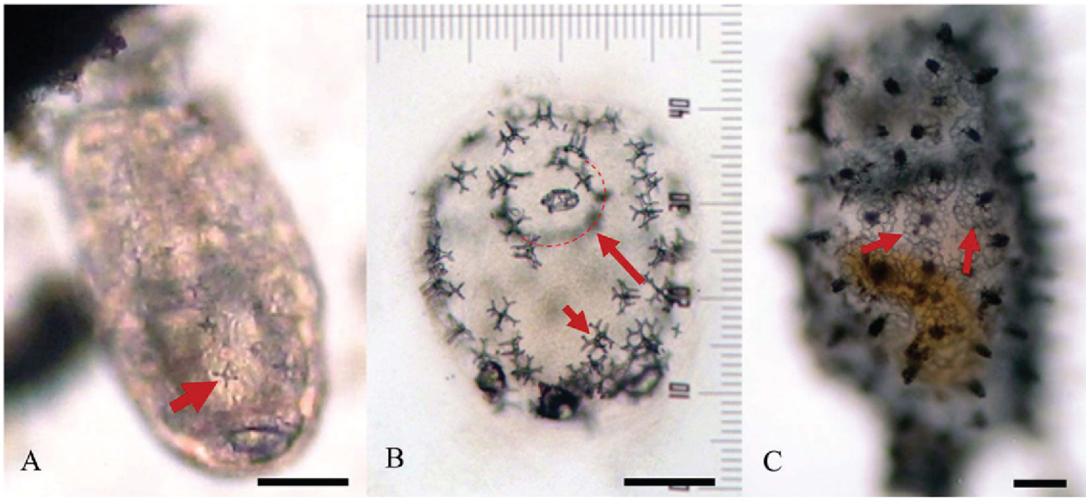

FIGURE 8.14

Ossicle development in *A. japonicus*. (A) 13-Day-old pentactula with X-shaped ossicles (arrow). (B) 16-Day-old larva with calcareous ring ossicles (long arrow) and developed X-shaped ossicles (short arrow). (C) 25-Day-old juvenile with T-shaped ossicles surrounding the whole body (arrow). Scale bars = 100 μm.

From Qiu, 2013

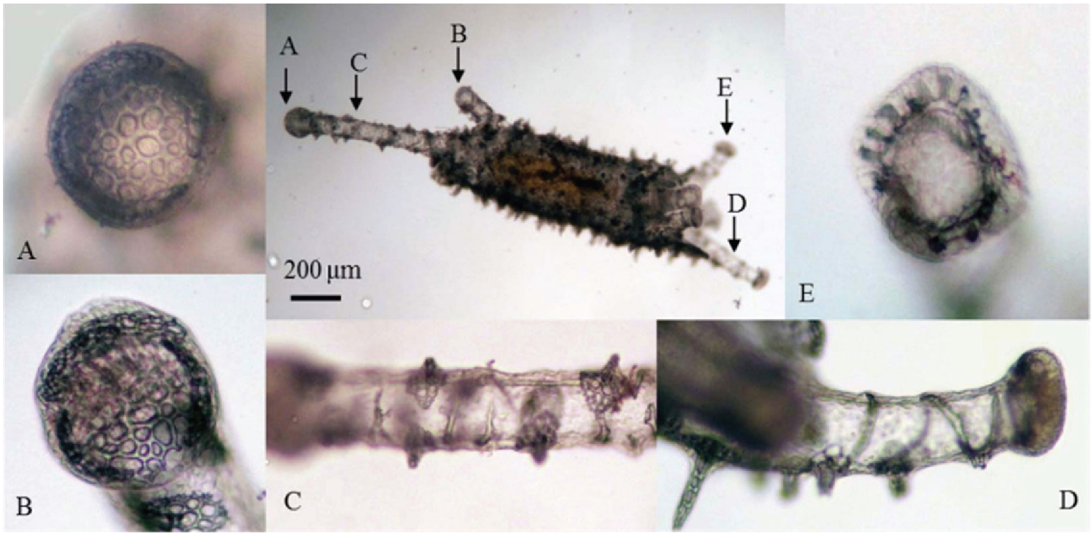

FIGURE 8.15

Juvenile of *A. japonicus* and its main locomotive organs. (A) Sucker of first ambulacral podium. (B) Sucker of second ambulacral podium. (C) Stalk of ambulacral podium. (D) Stalk of buccal podium/tentacle. (E) Sucker of buccal podium/tentacle.

From Qiu, 2013

As juveniles develop, the buccal tentacles gradually branch out and lose their ability to attach to the substrate. They consequently develop into a strictly feeding organ and lose their locomotory function (Figure 8.16). The colloid tissue that covers the buccal tentacles is a useful means of capturing food particles (Figure 8.16D). As buccal tentacles ramify, the number of ambulacral podia increases, and the sea cucumbers' locomotory ability is enhanced. The C-shaped ossicles (Figure 8.17, left) persist in the buccal tentacles as they develop into feeding organs, while in the ambulacral podia the T-shaped ossicles disappear, to be replaced by I-shaped ossicles (Figure 8.17, right), which enhance the podia's flexibility.

8.7 INTERINDIVIDUAL VARIATION IN GROWTH

Interindividual variations in growth rates, also known as "interspecimen size heterogeneity" (Salas-Leiton et al., 2011), is a common phenomenon found in many organisms under intensive production. Among these many species, fishes and holothuroids are of special concern because of their extremely high coefficient of variation (CV) on mean body weights (Wang et al., 1998; Huss et al., 2007; Merino et al., 2007; Liang et al., 2010; Pei et al., 2012), which is usually more than 20% in fishes and 50% in holothuroids (Dong et al., 2009). The CV on body weight of the sea cucumber *A. japonicus* within a cohort can reach up to 105–365% (mean 243%) based on measuring 1200 haphazardly chosen samples from eight cohorts after six months of growth (Tianlong Qiu, unpublished data). Such large body size variation in sea cucumbers translates into additional work needed to grade the seedlings and in delayed production because of "production tails" or "slow growers."

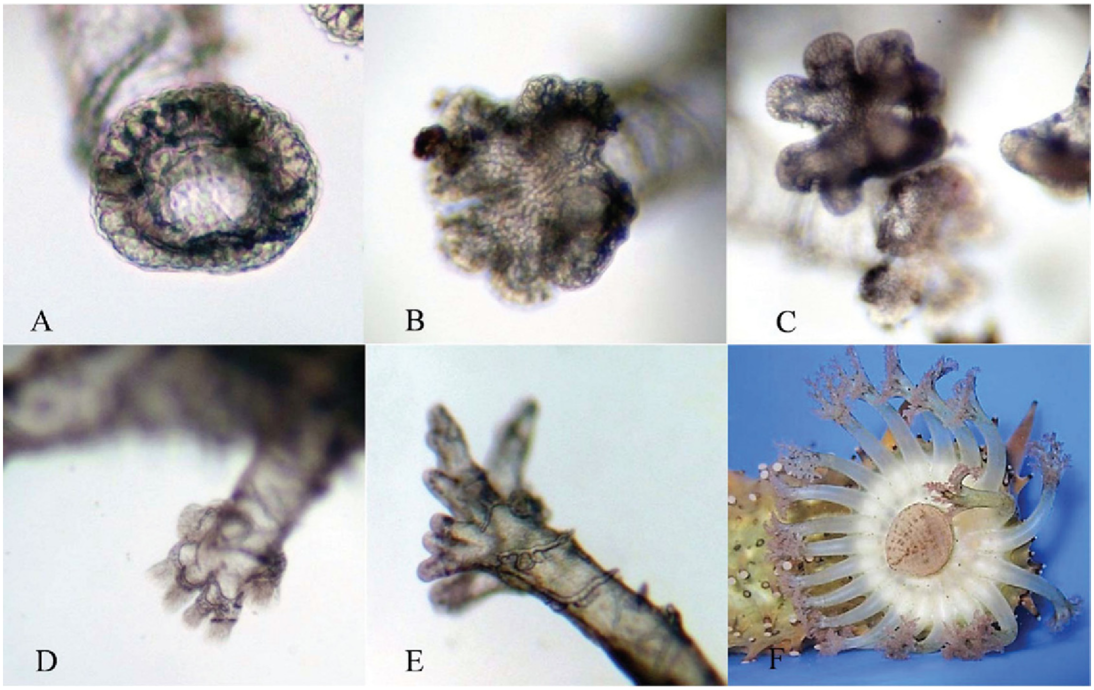

FIGURE 8.16

Development of buccal podia/tentacles in *A. japonicus.* (A) First buccal podium of a 25-day-old juvenile with one ambulacral podium. (B–C) Buccal tentacle of 36-day-old juvenile with 4–5 ambulacral podia. (D) Buccal tentacle of 45-day-old juvenile (showing the colloid tissue). (E) Buccal tentacle of 60-day-old juvenile. (F) Buccal tentacles of two-year-old adult.

From Qiu, 2013

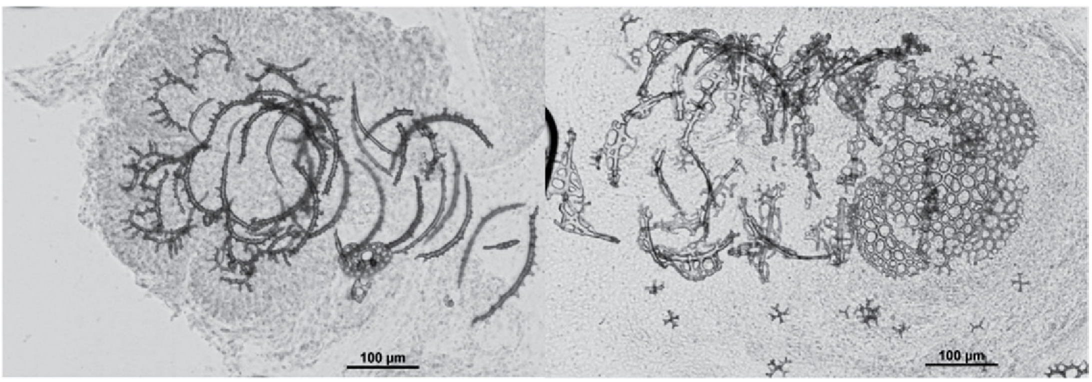

FIGURE 8.17

Ossicles in *A. japonicus* individuals bearing 10 ambulacral podia (60 days old). Ossicles of buccal tentacle (left) and ambulacral podium (right).

From Qiu, 2013

The mechanism underlying growth variations in fishes is thought to be associated with inherent factors, such as maternal effects, and environmental factors, such as the interaction between individuals, unequally distributed resources, and environmental heterogeneity (Bernardo, 1996; Johnston and Leggett, 2002; Huss et al., 2007). Competition for living resources within cohorts was thought to be the main factor responsible for growth variations in salmon (Davis and Olla, 1987; Nakano et al., 1991). Sloman (2007) emphasized that environmental conditions experienced by early stages strongly influenced behavioral and physiological traits in salmon, and that this would lead to differences in acquisition of food at first feeding, which resulted in interindividual growth variations. Other research in fishes and holothuroids, including on *A. japonicus*, suggested that inherent factors may play a leading role (Wang et al., 1998; Martins et al., 2005; Liang et al., 2010). Stocking density was also thought to be an important mediator of growth, and consequently of the CV of animal size, by affecting environmental factors; at higher stocking densities buildup of nitrites could reduce the quality of the surrounding water (Irwin et al., 1999; Huss et al., 2007).

Dong et al. (2009) and Liang et al. (2010) inferred that genetic differences within cohorts largely accounted for interindividual growth variation in the sea cucumber *A. japonicus*, while stocking density also played a role. Further study by Pei et al. (2012) indicated that both genetic and behavioral factors played key roles in growth variation, but that chemical mediation could not be ruled out. However, data in support of a genetic control are still insufficient and the potential influence of culture methods on growth/size variation remains unclear.

Qiu et al. (2014) also argued that interindividual growth variation was mostly due to genetic factors, i.e., differences in food conversion efficiency (FCE) among individuals. However, the level of growth variation could be influenced by the level of contacts among individuals and environmental heterogeneity resulting from different culture methods (e.g., stocking density). The study suggested that, apart from using traditional grading methods, designing a new type of substrate might be warranted, which can change the spatial distribution of sea cucumbers and may effectively enhance growth and reduce interindividual variability to some extent.

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CHAPTER

ENVIRONMENTAL DRIVERS OF BEHAVIOR

9

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SUMMARY

Environmentally driven behaviors are those that are mediated by environmental cues, such as temperature, salinity, and light. This chapter explores environmentally driven behavior in embryos, larvae, juveniles, and adults of *Apostichopus japonicus*. It covers their movement, migration and aggregation, timing of spawning, as well as growth and feeding patterns. It also outlines the factors that affect habitat selectivity, migration, and distribution of *A. japonicus* at different periods. Knowledge of how behaviors of *A. japonicus* are mediated by environmental factors is the basis for developing adapted facilities and strategies for their culture and stock enhancement, as well as better management and protection of the remaining natural populations.

Keywords: aestivation; *Apostichopus japonicus*; behavior; environmental drivers; hibernation; salinity; sea cucumber; spawning; temperature

9.1 DAILY ACTIVITY PATTERNS

Daily activity patterns refer to movements (including migration and aggregation) that can be detected at scales ≤24 h. Studies of these patterns in early life stages of *Apostichopus japonicus* remain relatively rare. This section examines movements in embryos and larvae of *A. japonicus*, including behavior and vertical distribution, and how these can be affected by reduced salinity of surface seawater under laboratory conditions.

9.1.1 PATTERNS IN THE MOVEMENTS OF EMBRYOS AND LARVAE

Kashenko (2002) studied vertical distributions in early stages of *A. japonicus* (blastula, late gastrula, early auricularia, 10-day-old auricularia, late auricularia with hyaline spheres, and doliolaria) during dilution experiments. Embryos/larvae were placed at a depth of 19–20 cm in 1-L cylinders (50 cm depth, 6 cm diameter) at an ambient salinity of 32. Surface water was then diluted by gently adding water of reduced salinity to create a stable density gradient that was either sharp (ambient seawater topped with a layer of freshwater) or gradual (ambient seawater topped with a layer at 20 and another layer at 12). Salinities and densities of embryos/larvae were measured in various horizons over time and compared to an undiluted control.

Zhang, L., Pan, Y., Song, H. 2015. Chapter 9. Environmental drivers of behavior. In: Yang, H., Hamel, J.-F., Mercier, A. (Eds.), The Sea Cucumber *Apostichopus japonicus*. History, Biology and Aquaculture, Academic Press, pp. 133–152. http://dx.doi.org/10.1016/B978-0-12-799953-1.00009-X

Kashenko (2002) found that blastulae (at 16 h, 195 ± 24 μm) were moving freely up and down the water column under normal salinity conditions, with the highest densities found in the surface layer. When salinity was reduced to 9.5–13 in the surface layer, blastulae accumulated in the underlying horizon at a salinity of 20–20.5. They lost motility after being in contact with water of reduced salinity because of osmotic shock, and increased in size as a result of hydration but they recovered locomotor activity and survived, although they were not able to leave the diluted horizon.

Late gastrulae (31 h) nearing transition to the larval stage also moved up and down and were uniformly distributed in the water column (excluding the top 0–1 cm layer) under normal salinity (Kashenko, 2002). However, once a diluted surface layer (salinity of 6.5–17) had formed, late gastrulae began to congregate in the horizon with a salinity of 20.0–20.5 where their density reached 17.4 ± 1.8 ind. mL^{-1} after 1 h. Their ciliary movements and hydration were visibly affected. However, after 3 h, their condition improved and they began leaving the diluted layer. Concurrently, embryos in control cylinders descended to a depth below 10 cm.

The experiments of Kashenko (2002) showed that the movements of larvae can be influenced by salinity changes. The auricularia larva is the longest-lived planktonic stage in *A. japonicus*. Early auriculariae (61 h, 538 ± 29 μm), mid-auriculariae (10 d, 925 ± 43 μm), and late auriculariae (with hyaline spheres; 763 ± 35 μm) showed the same pattern of activities in the control vessels (salinity of 32). They were moving in large groups upward and downward, and were uniformly distributed in the water column, but not entering the uppermost 0–1 cm layer. In treatment groups with a surface salinity of 5–15.5, early auriculariae migrated deeper toward the salinity horizon of 31–32 and did not enter the diluted uppermost layers. Mid-auriculariae concentrated in the layer with a salinity of 20, where their density was 13.02 ± 0.24 ind. mL^{-1} after 1 h. Kashenko (2002) mentioned that larvae could not leave the horizon at a salinity of 20 because of the osmotic shock, but did leave the layer at 25–26. In dilution trials, late auriculariae, which were small and more motile, also migrated deeper (to a depth of 18 cm) where salinity was 32. Doliolaria larvae remained near the bottom under control conditions, with few larvae approaching the surface. They actively migrated away from the diluted surface salinity (5–15) to a depth of 17–18 cm where normal salinity occurred.

While information on daily vertical migrations of sea cucumber larvae in the natural environment are not available, the study of Kashenko (2002) recorded upward and downward migrations of larvae under control conditions that may reflect their behavior in still water in the wild. Results also showed that larval stages were better equipped to escape and move away from the diluted upper layer in non-turbulent water.

9.1.2 MOVEMENT, MIGRATION, AND AGGREGATION OF JUVENILES AND ADULTS

The sea cucumber *A. japonicus* is a cryptic animal that shelters by day and emerges at night. Kato and Hirata (1989) outlined two types of circadian rhythms in *Apostichopus* (as *Stichopus*) *japonicus* from laboratory work in Japan. Unimodal circadian rhythms with a peak at 02:00 were found at temperatures between 18 and 25 °C, while bimodal circadian rhythms with two peaks (at 14:00 and 22:00) were observed at lower temperatures, between 12 and 17 °C. They thought that bimodal circadian rhythms might be related to feeding activity and higher metabolic rates at lower temperatures. More movement (49.6 m day^{-1} on average) was recorded at temperatures <17 °C, and less (21.6 m day^{-1} on average) at temperatures >18 °C.

Dong et al. (2011) studied the effects of photoperiod on the daily activity rhythms of juveniles of *A. japonicus* (weighing 29.73 ± 0.23 g). They used nine treatments of light:dark (LD) cycles

(0:24, 3:21, 6:18, 9:15, 12:12, 15:9, 18:6, 21:3, and 24:0) and measured the observed distribution rates (DR),

$$\mathrm{DR} = \sum_{i}^{n}\left(\frac{Mi}{nN}\right)\times 100\%$$

The activity rhythms of *A. japonicus* under the different photoperiods (Figure 9.1) show that juvenile sea cucumbers have distinct rhythms; most were retreating to shelters during daytime and emerging and feeding during nighttime. The study also found that there was no difference in mean DR per day under LD cycles of 6:18, 9:15, 15:9, and 12:12. Under these photoperiods, mean DRs per day were lower than those under LD cycles of 0:24 and 3:21, and higher than those under LD cycles of 18:6, 21:3, and 24:0. These results were possibly influenced by some internal physiological clock and the even daily light variation.

Compared to the LD cycles that had more than 6 h of light (9:15, 12:12, 15:9, 18:6, 21:3, and 24:0), the 0:24 cycle yielded sea cucumbers that spent less time sheltering during the "daytime" and more time feeding at "nighttime." But these sea cucumbers spent more time sheltering and less time feeding than those under LD cycles of 3:21 and 6:18.

The behavioral transition processes of *A. japonicus* typically occurred at "dawn" and "dusk" (Figure 9.1). There were significant sheltering behavior transitions at dawn between 03:00 and 06:00 under all photoperiods, but in the 0:24 cycle, sea cucumbers retreated to the shelters from 03:00 to 09:00 (when the DR decreased from 82.4 to 64.8%) in a more gradual process (Figure 9.1). There were also significant emerging behavior transitions at dusk. The authors found that there was a single emerging behavior transition under LD cycles of 0:24 and 24:0, corresponding to the "dusk time" (from 15:00 to 21:00 and from 18:00 to 21:00, respectively) (Figure 9.1), and two emerging behavior transitions under cycles of 3:21, 6:18, and 9:15, one corresponding to the end of illumination and one to "dusk" (Dong et al., 2011).

Figure 9.2 provides a different illustration of the mean DR of juveniles outside the shelters during the light and dark periods under different photoperiods. The DR in both light and dark intervals initially decreased and then increased again as day lengths in the treatments increased. However, the trends of DR across different photoperiod treatments differed between the light and dark intervals. For "light time," the DR varied as follows: LD cycles 21:3 > 24:0 > 3:21 > 15:9 > 18:6 > 12:12 > 9:15 > 6:18, whereas for "dark time," the DR varied as follows: 0:24 > 3:21 > 21:3 > 15:9 > 6:18 > 9:15 > 12:12 > 18:6 (Figure 9.2).

Furthermore, the daily activity of juvenile *A. japonicus* was affected by light intensity according to Zhang et al. (2006), who studied light intensity's effect on the behavior as well as the effect of artificial reef attractiveness under laboratory conditions. Dark controls were compared with illumination provided by incandescent lamps (15–60 W). They found that for sea cucumbers weighing 145 ± 0.5 g, illumination below 10 lx was more suitable. Zhang et al. (2006) used distribution rate (DR; shown earlier), and mean attraction rate (MAR):

$$\mathrm{MAR} = \sum_{i}^{n}\left(\frac{Ni}{nN}\right)\times 100\%$$

to describe the distributions of *A. japonicus* under the different light conditions tested (Figure 9.3).

In environments without artificial reef but exposed to light, most sea cucumbers stayed in low-light areas, but this behavior was not consistent. In environments offering both reef and light, most sea cucumbers gathered inside the artificial reef or in the shadow created by it. Sea cucumbers kept in the dark

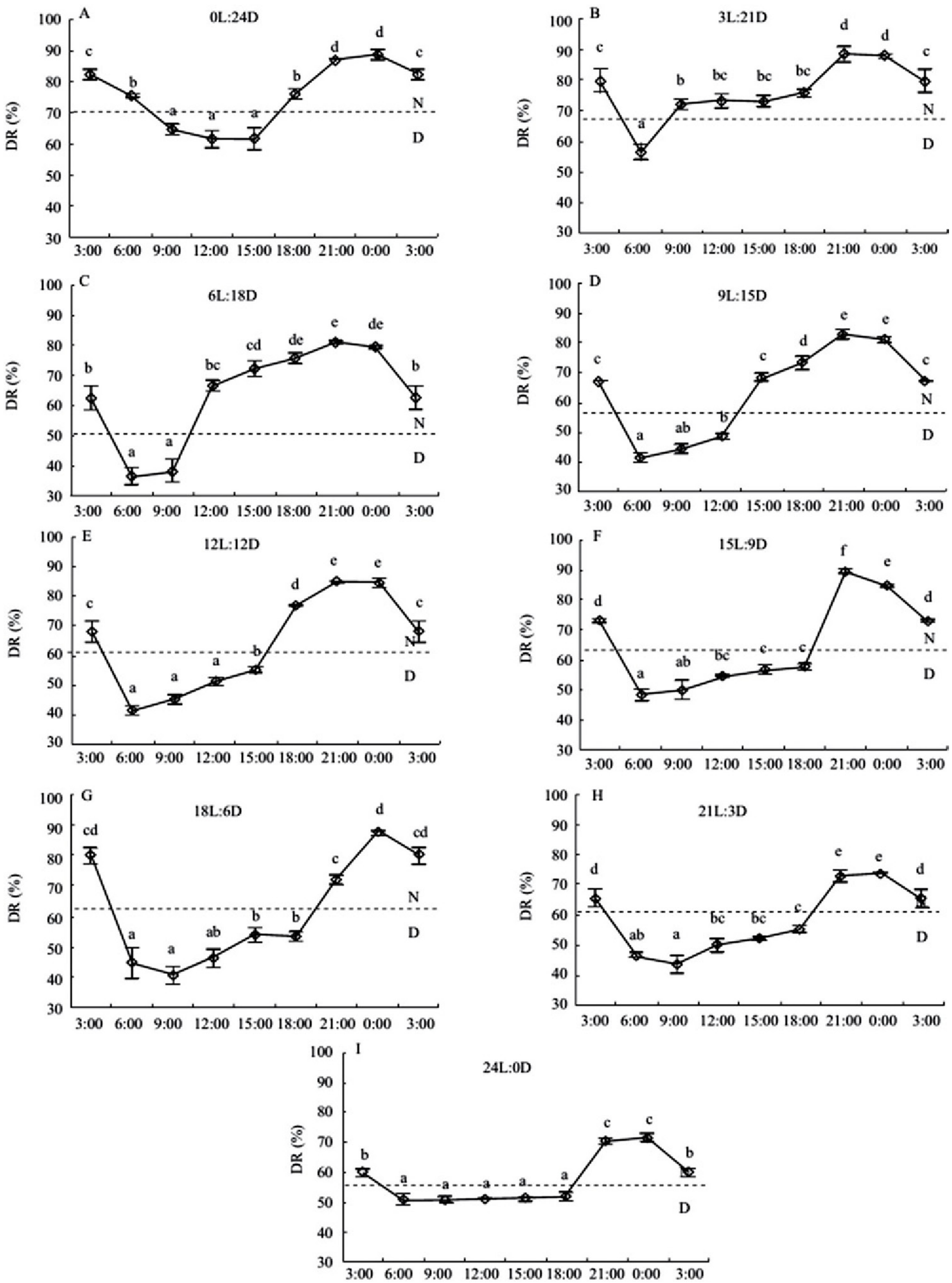

FIGURE 9.1

Daily activity rhythms of *A. japonicus* as mean distribution rates (DR) over time under different photoperiods. Different letters indicate significant differences ($P < 0.05$), and bars represent standard errors of means. The letters "D" and "N" represent "daytime" and "nighttime," respectively. The dotted lines were drawn based on visual cues for "daytime" (D) and "nighttime" (N) from the viewpoints of the animal behaviors and they were set according to the behavior transition processes.

From Dong et al., 2011

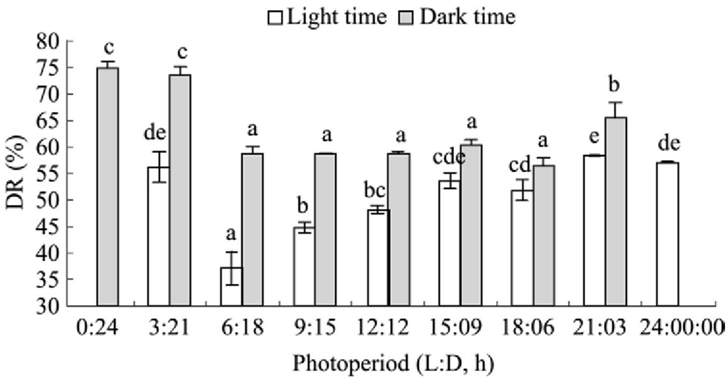

FIGURE 9.2

Mean distribution rates (DR) of *A. japonicus* in the light and dark periods under different photoperiods. Different letters indicate significant differences ($P < 0.05$), and bars represent standard errors of means.

From Dong et al., 2011

or under lower light intensity (<10 lx) exhibited intense activity (moving and feeding), and fewer gathered in the artificial reef or shaded areas. This suggests that *A. japonicus* prefers habitats with low light intensity (Zhang et al., 2006). In addition, sea cucumbers exhibiting a dark green color avoided strong light irradiation, and quickly moved to avoid direct exposure to light, whereas sea cucumbers exhibiting a light green color tended to be more resistant to strong light irradiation. An extreme example was provided by Lin et al. (2013) who compared the effects of light intensity on movement and growth of albino and normal *A. japonicus*. The mean probability distribution (MPD, %) was used to show the distribution of *A. japonicus* at a given position on the grid and was calculated with the following equation:

$$\text{MPD}(\%) = \frac{1}{n}\sum_{t}^{n}\frac{qx_i y_j}{S}\times 100\%$$

Where qx_iy_j refers to the quantity of the same type of sea cucumber in each cell; S the sum of the same type of sea cucumber; i and j the coordinates of the cell; t the time of the experiment; and n the total number of times the experiment was repeated. They found that both albino and normal specimens had negative phototaxis, but normal *A. japonicus* were more responsive to intense light than albinos, and the latter could withstand longer periods of exposure to intense light. Figure 9.4 illustrates the distribution of illumination values, whereas the migrations of albino and normal green sea cucumbers are shown in Figures 9.5 and 9.6, respectively. The results showed that normal green sea cucumbers moved more quickly to the low-light area after being placed in the center of the flume than albino sea cucumbers.

Dong et al. (2010a) studied the activity of *A. japonicus* (30.27 ± 3.08 g) under eight light-intensity treatments (natural light, continuous darkness, and 15, 30, 60, 125, 250, and 500 lx under LD 12:12 cycle). They showed that there are cyclic nocturnal activity patterns of behavior at different light intensities in the range of 15–500 lx under the 12:12 cycle. *A. japonicus* sheltered in the daytime and moved at nighttime under natural daylight and ambient LD cycle (Figure 9.7). Only a small proportion of individuals (26.15 ± 2.96%) were exposed to light between 06:00 to18:00 with some retreating to shelters between 06:00 and 09:00. But during nighttime (21:00–03:00), a large number of individuals (58.64 ± 4.55%) emerged, with a peak emergence at 00:00 (64.35 ± 5.79%).

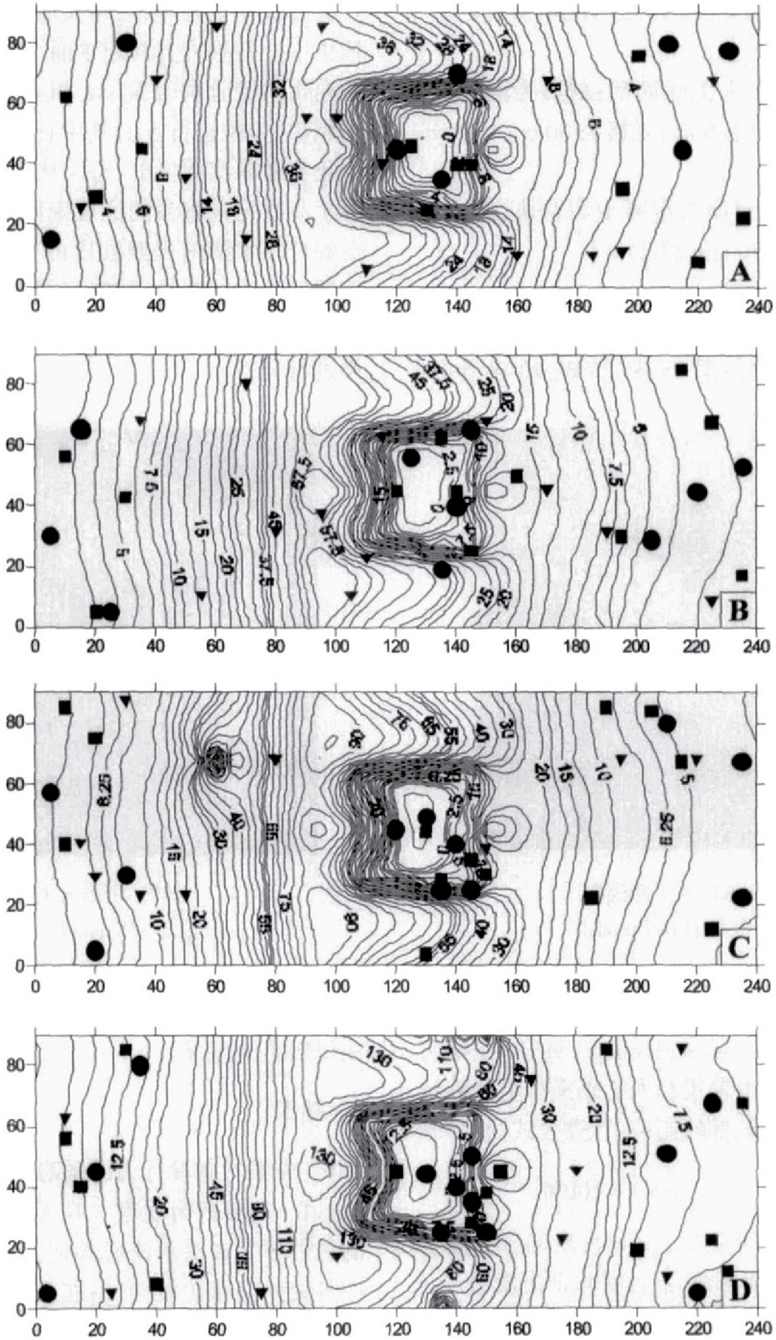

FIGURE 9.3

Distribution of *A. japonicus* under different light intensities (water temperature = 13.5 ± 0.5 °C, dissolved oxygen >6.0 mg L^{-1}, pH = 7.8–8.2, and salinity = 34 ± 0.5). Horizontal and vertical axes indicate the length and width of the water flume separately (in cm). The number on isolines shows light intensity (in lux). Panels A–D correspond to light intensities of 15, 25, 40, and 60 W, respectively; symbols ▲, ■, ● correspond to *A. japonicus* locations, showing DR < 20%, 20% < DR < 50%, and DR > 50%, respectively.

From Zhang et al., 2006

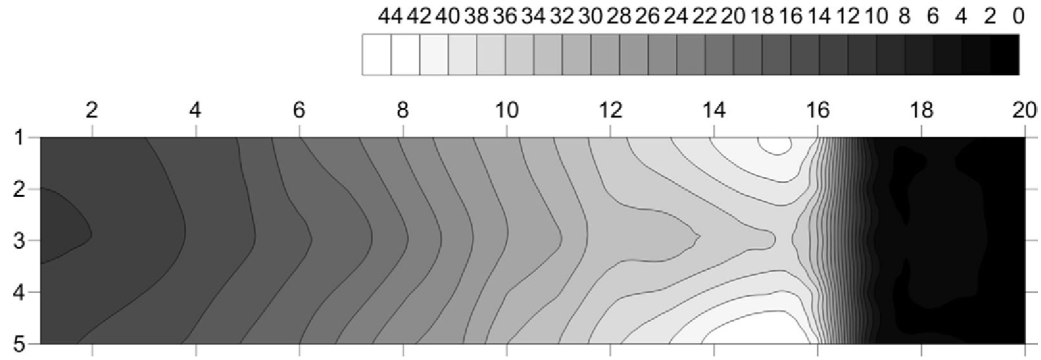

FIGURE 9.4

Light gradient in an experimental flume tank. Illumination for the experiment was provided on one side of the tank using a 300 W tungsten iodine lamp and light intensity measured using a ZDS-10F-2D digital underwater luxmeter. Light intensity values (lx) are shown on the upper scale. The *x* and *y* axes correspond to the size of the flume tank (×10 cm).

From Lin et al., 2013

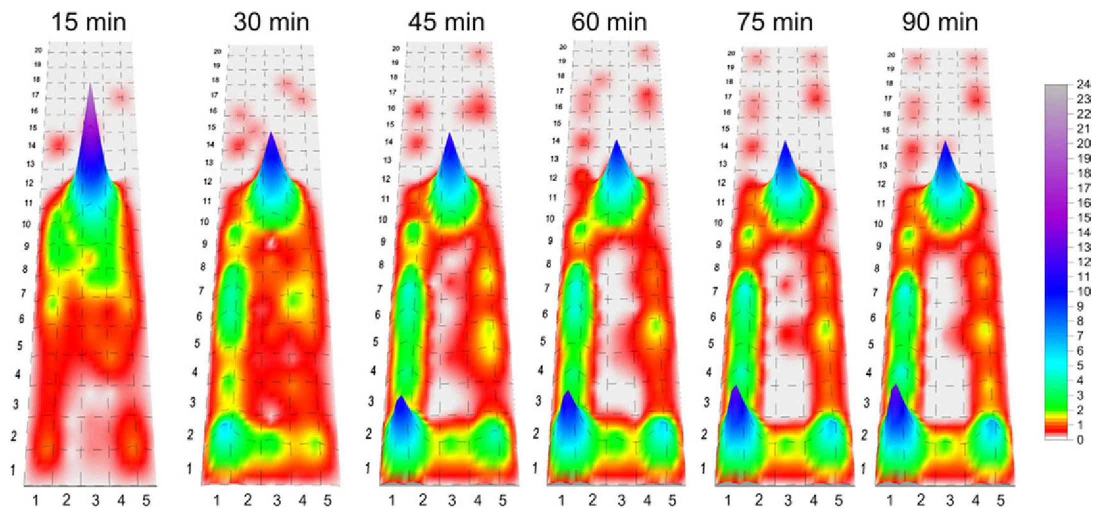

FIGURE 9.5

Distribution of albino *A. japonicus* at 15-min intervals over the first 90 min of an experiment testing the effect of light intensity on their behavior. The scale of mean probability distribution (MPD, %) is shown on the right of the figure. The *x* and *y* axes correspond to the size of the flume tank (×10 cm). Light intensity gradient in the flume tank is shown in Figure 9.4.

From Lin et al., 2013

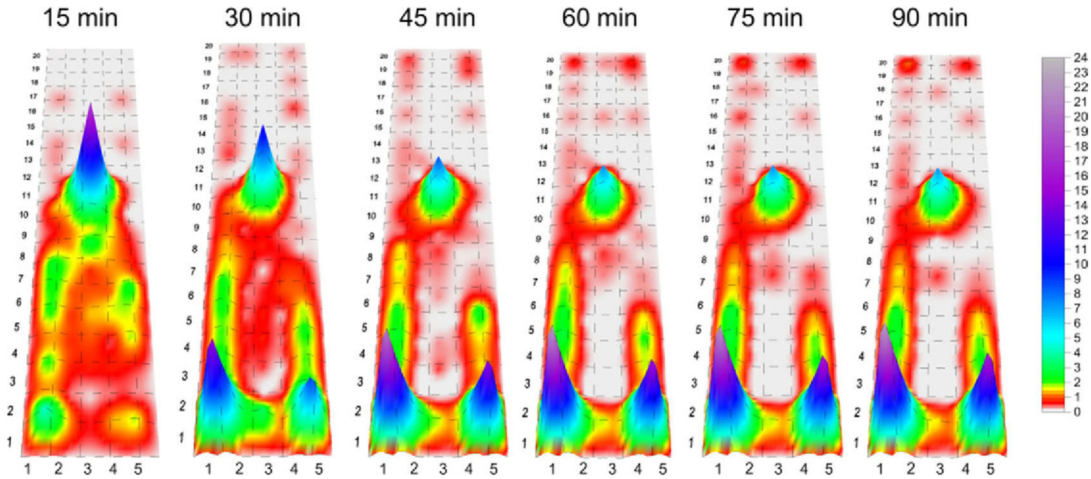

FIGURE 9.6

Distribution of normal green *A. japonicus* at 15-min intervals over the first 90 min of an experiment testing the effect of light intensity on their behavior. The scale of mean probability distribution (MPD, %) is shown on the right of the figure. The *x* and *y* axes correspond to the size of the flume tank (×10 cm). Light intensity gradient in the flume tank is shown in Figure 9.4.

From Lin et al., 2013

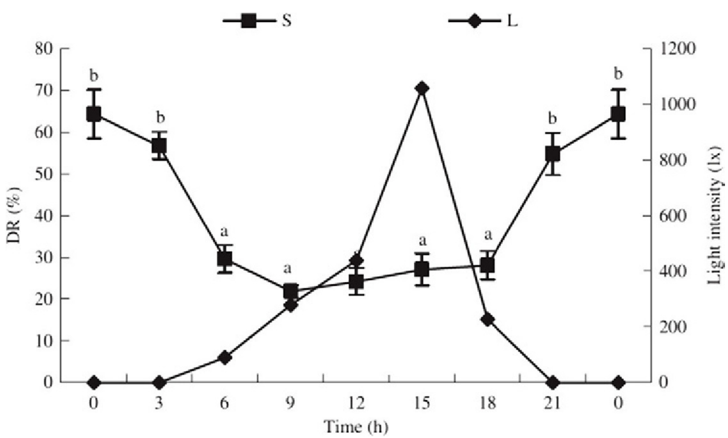

FIGURE 9.7

Daily activity rhythm based on distribution rates (DR) of *A. japonicus* under natural light. S and L stand for the daily activity cycle of *A. japonicus* and the light intensity, respectively. Different letters indicate significant differences ($P < 0.05$), and the values are provided as mean ± SE ($n = 3$).

From Dong et al., 2010a

Dong et al. (2010b) used an exposure index (EI) to describe the distribution in the different light intensity treatments. EI is the percent number of *A. japonicus* individuals observed outside the shelters on the light side of the tank divided by the total number of individuals. With the increase in light intensity, the daily activity cycle of *A. japonicus* was more obvious (Figure 9.8). At 15 lx, there was a clear difference in the EI between daytime (06:00–15:00) and evening hours (18:00–00:00), but there were no differences in EI at other times. When light intensity was >30 lx, there were differences in EI between the 06:00–15:00 interval and all the other times. Under strong light (250 and 500 lx), the EI of *A. japonicus* was below 10% and only a few individuals were ever seen to be exposed on the bright side of the tank (Figure 9.8).

Dong et al. (2010a) also studied the effects of materials, incubation time, and colors of artificial shelters on the behavior of juveniles of *A. japonicus*. Daily rhythms in the number of sea cucumbers attracted by artificial shelters were found (Figure 9.9). The green, red, and blue shelters seemed to attract slightly more sea cucumbers, although statistical differences were not found (Figure 9.10).

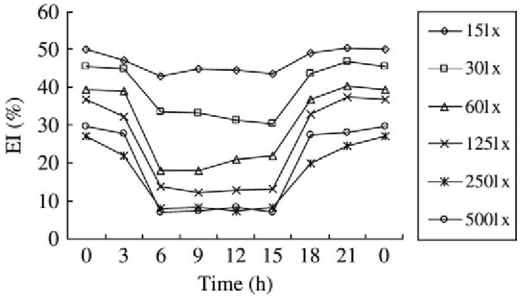

FIGURE 9.8

Daily activity rhythm of *A. japonicus* as exposure index (EI) over time under different light intensities, from 15 to 500 lx.

From Dong et al., 2010a

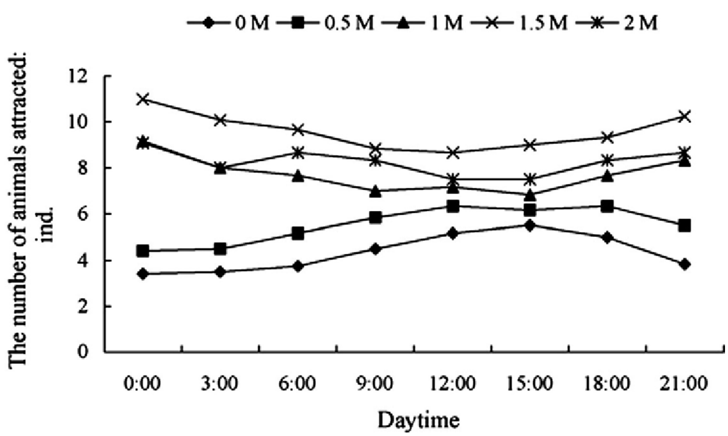

FIGURE 9.9

Daily activity rhythm of *A. japonicus* attracted by shelters. *Y* axis shows the number of animals attracted by shelters. Month was abbreviated to M. 0 M stands for nonincubation, 0.5, 1, 1.5, and 2 M stand for incubation for 0.5, 1, 1.5, and 2 months, respectively.

From Dong et al., 2010b

Zhang et al. (2006) showed that the attractiveness of artificial reef structures was related to the shade they offered under different light intensities (Figure 9.3). Mean attraction rates (MARs) in treatments of 15 and 25 W light intensity were lower compared to the 60 W treatment, when using both cubic and pyramidal reefs (Figure 9.11). The cubic reef was made of concrete and measured about 30 × 30 × 30 cm, with one square-shaped opening of about 10 × 10 cm on each surface. The pyramidal

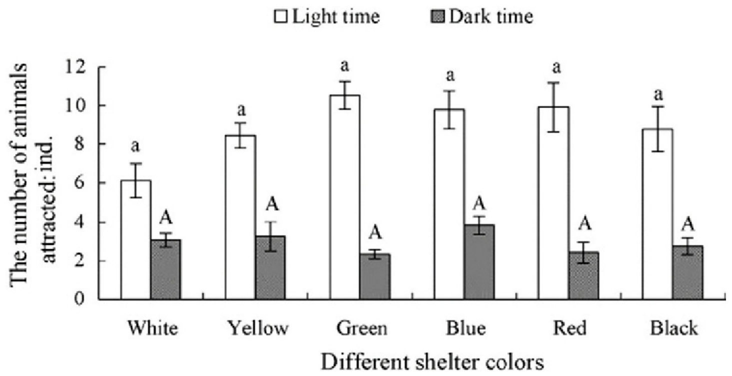

FIGURE 9.10

Number of *A. japonicus* attracted by shelters of different colors. *Y* axis shows the number of animals attracted by shelters. Different letters indicate significant differences ($P < 0.05$), and bars represent standard errors of means (Sample size $n = 90$).

From Dong et al., 2010b

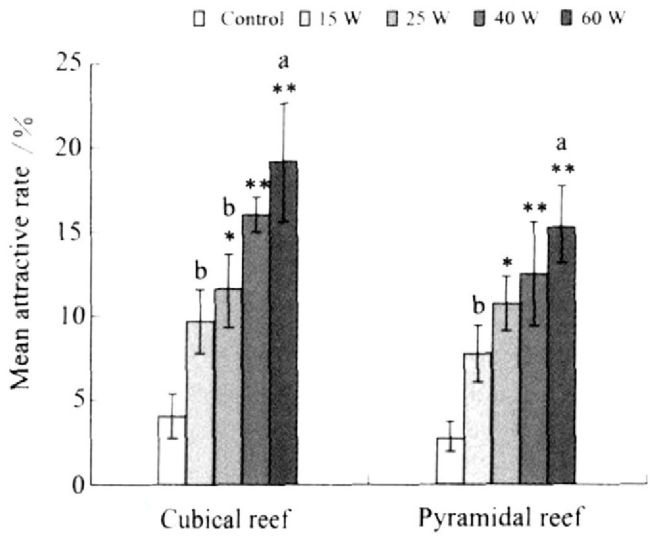

FIGURE 9.11

Effects of four different incandescent lamps on mean attraction rate to the two types of reefs tested (cubic reef and pyramidal reef). * Significantly different compared with control ($P < 0.05$); ** very significantly different compared with control ($P < 0.01$). Letters identify significantly different treatments ($P < 0.05$).

From Zhang et al., 2006

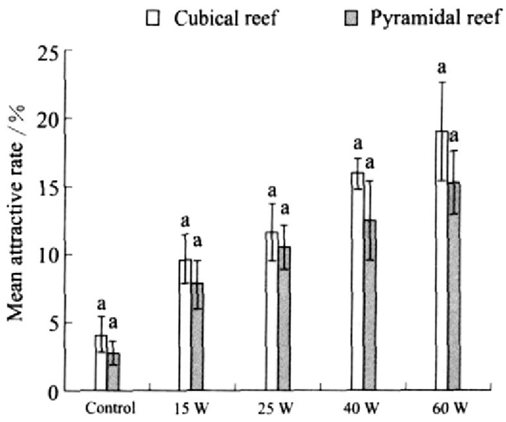

FIGURE 9.12

Comparisons of mean attractive rate (MAR) of juvenile *A. japonicus* on two types of reefs (cubic reef and pyramidal reef) tested under different light intensities. Different letters identify significant differences between groups ($P < 0.05$).

From Zhang et al., 2006

reef was also made of concrete, with a bottom square measuring 30 × 30 cm and a tip raised at about 30 cm high. Under the same condition, MARs were not different between the two types of artificial reefs (Figure 9.12).

9.1.3 FEEDING PATTERNS

Daily fluctuations in environmental conditions can affect feeding behaviors and ultimately influence growth patterns.

Chen et al. (2007) tested the growth of juveniles under various light regimes: 24 h light, 24 h dark, 12 h light:12 h dark, and natural photoperiod. The best growth rate was recorded when photoperiod was adjusted at 12:12, and the lowest growth rate occurred under 24 h light (Figure 9.13).

Research by Dong and Dong (2006a) on juvenile *A. japonicus* found that constant temperatures (12, 15, 18, 21, or 24 °C) and daily fluctuating temperatures (15 ± 2, 18 ± 2, and 21 ± 2 °C) had different effects on growth as well as proximate body composition and oxygen consumption. Sea cucumbers were reared in a fluctuating temperature control system (Figure 9.14) with conditions shown in Figure 9.15. At different constant temperatures, growth of juveniles varied, displaying a maximum specific growth rate (SGR) of 1.48% day^{-1} at 16–18 °C. Growth was significantly lower at 24 °C than at any of the other four constant temperatures tested. Furthermore, growth rates were enhanced at temperatures that fluctuated (15 ± 2 °C and 18 ± 2 °C) compared to the corresponding constant temperatures (15 and 18 °C). However, growth rates decreased under fluctuating conditions at a mean temperature of 24 °C (Table 9.1).

It was demonstrated by Dong and Dong (2006b) that the body crude lipid content increased with final body weight reached, both under constant and fluctuating temperature conditions. Overall, crude lipid contents under fluctuating temperatures were lower than those under the corresponding constant temperatures. The highest body crude lipid content was obtained at a mean temperature of 15 °C under both conditions. Some differences were also observed in tissue composition, such as moisture, protein, ash, and energy contents (Dong and Dong, 2006a; Figure 9.16). Ash content was higher under constant

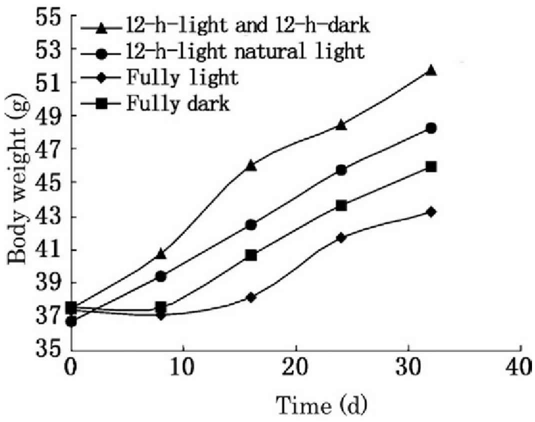

FIGURE 9.13

Weight increments in *A. japonicus* under different light regimes.

From Chen et al., 2007

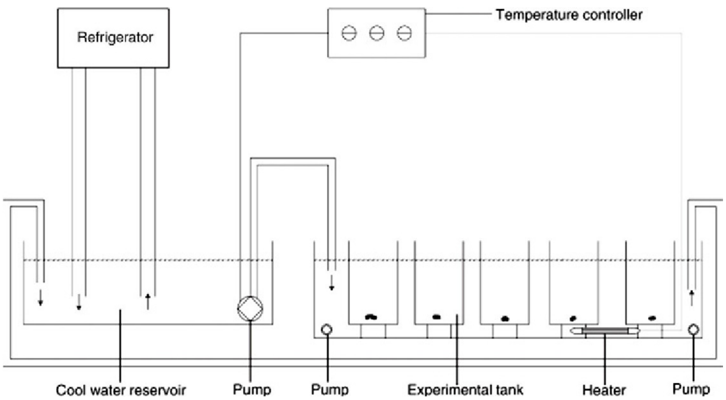

FIGURE 9.14

Diagram showing fluctuating temperature system used by Dong and Dong. Fluctuating temperatures were obtained using programmed temperature controllers that mixed heated and cool water. Arrows indicate the directions of water flow.

From Dong and Dong, 2006a

temperature conditions at 15 °C than fluctuating temperature conditions at 15 (±2) °C, but higher under fluctuating conditions at 18 (±2) °C than constant temperature conditions of 18 °C (Figure 9.16d). Energy content was higher under varying conditions at 21 (±2) °C than at 21 °C, and higher under constant conditions at 18 °C than at 18 (±2) °C (Figure 9.16e).

Oxygen consumption rates increased with increasing temperature among the three constant temperature treatments, but there was no clear difference. Trends in the three fluctuating temperature treatments were similar, but oxygen consumption rates at 15 ± 2 °C was lower than at 21 ± 2 °C. The difference in

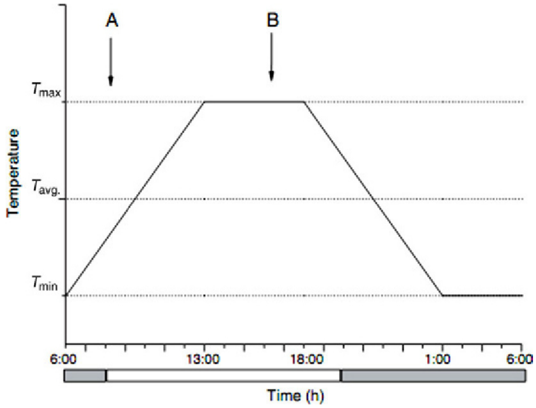

FIGURE 9.15

Diagram of the daily fluctuating temperature. Photoperiod regime is depicted by horizontal white (light period) and gray (dark period) bars. The arrows A and B represent the feeding times.

From Dong and Dong, 2006a

Table 9.1 Effects of Different Constant and Fluctuating Temperature Treatments on Growth of Juvenile *A. japonicus*

Treatments	Temperature (°C)	Initial Body Weight (g)	Final Wet Body Weight (g)	Final Dry Body Weight (g)	SGR* (% day^{-1})
Constant					
12	12 ± 0	9.44 ± 0.64^{a}	22.89 ± 1.59^{a,b}	1.78 ± 0.10^{a,c,f}	1.41 ± 0.08^{b}
15	15 ± 0	9.90 ± 0.47^{a}	25.50 ± 1.53^{a,c}	1.95 ± 0.08^{a,d}	1.48 ± 0.06^{b}
18	18 ± 0	9.46 ± 1.35^{a}	26.31 ± 2.67^{c}	1.86 ± 0.21^{c,d}	1.48 ± 0.13^{b}
21	21 ± 0	8.54 ± 0.78^{a}	22.72 ± 2.56^{a,c}	1.62 ± 0.19^{c,e}	1.42 ± 0.12^{b}
24	24 ± 0	8.61 ± 1.71^{a}	15.53 ± 2.79^{f}	1.20 ± 0.22^{b}	0.99 ± 0.17^{c}
Fluctuating					
15 ± 2	15 ± 2	9.68 ± 0.79^{a}	31.82 ± 2.48^{d}	2.33 ± 0.29^{g}	1.76 ± 0.23^{a}
18 ± 2	18 ± 2	8.88 ± 0.27^{a}	29.92 ± 2.56^{d}	2.12 ± 0.25^{d,g}	1.74 ± 0.12^{a}
21 ± 2	21 ± 2	9.12 ± 0.67^{a}	17.20 ± 3.33^{b,c}	1.26 ± 0.21^{e,f}	0.96 ± 0.26^{c}

Values (mean ± S.E., n = 5) in the same column not sharing a common superscript letter are significantly different (P < 0.05).
**Specific growth rate = 100×[ln(final body weight) − ln(initial body weight)]/day.*
From Dong and Dong, 2006a

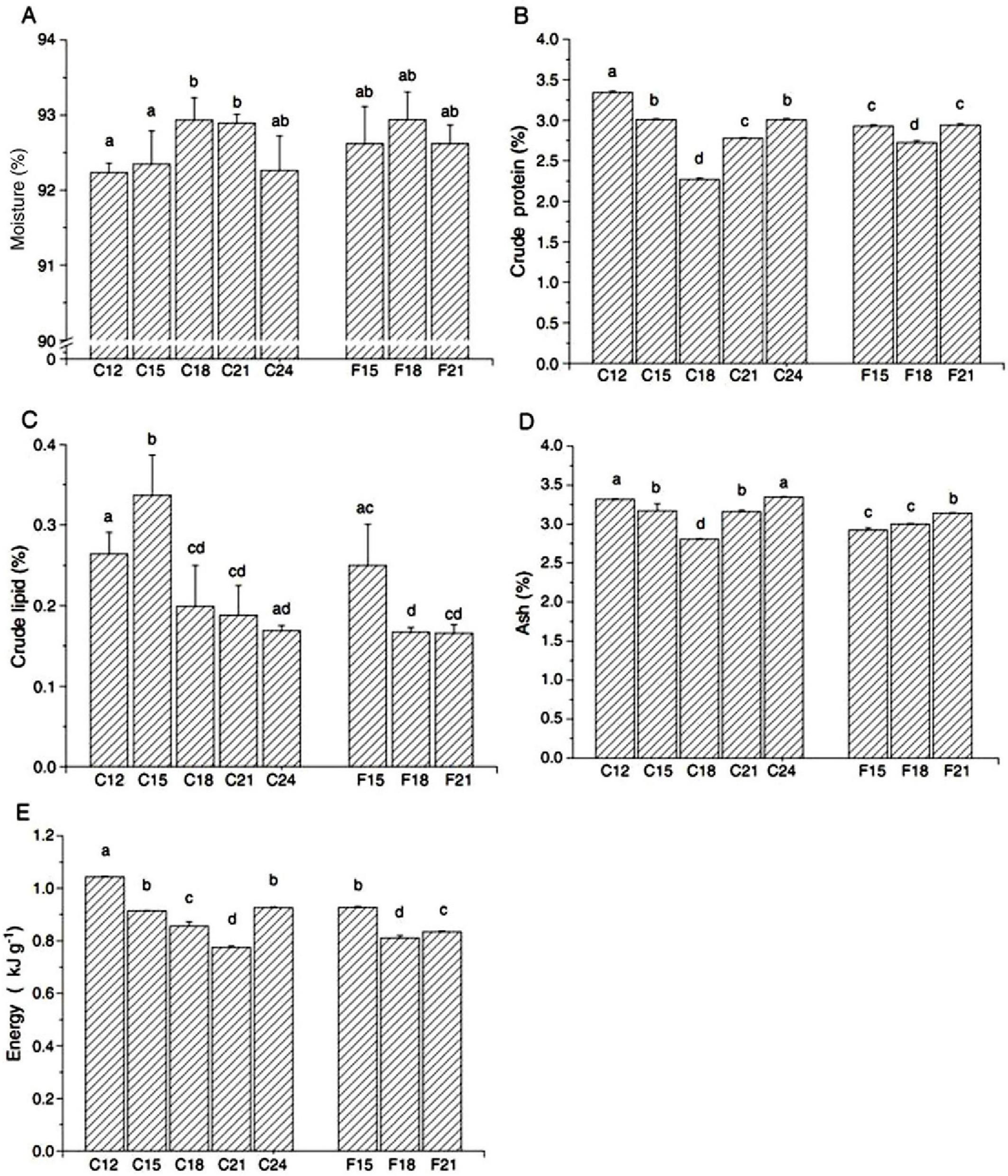

FIGURE 9.16

Effects of fluctuating temperatures on the proximate body composition of *A. japonicus* (A). Water content, (B) crude protein content, (C) crude lipid content, (D) ash content, and (E) energy content. Common letters denote no significant difference between means.

From Dong and Dong, 2006a

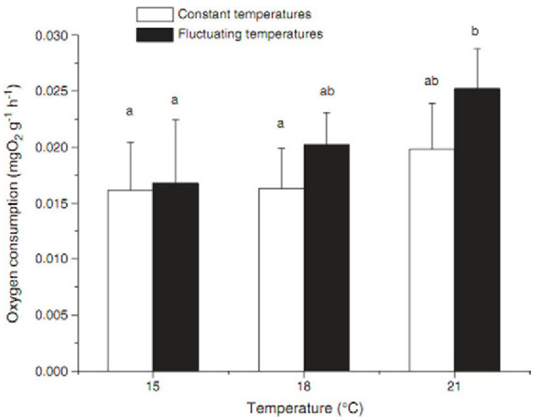

FIGURE 9.17

Oxygen consumption rates of *A. japonicus* under three fluctuating temperature regimes (15 ± 2, 18 ± 2, and 21 ± 2 °C) and three constant temperatures (15, 18, and 21 °C) (mean ± S.E.). Common letters denote no significant difference between means.

From Dong and Dong, 2006a

oxygen consumption rates between the fluctuating and the corresponding constant temperature treatments were not significant (Figure 9.17).

9.2 MONTHLY AND SEASONAL ACTIVITIES

Monthly and seasonal activity patterns refer to those that span more than a day and can be detected within an annual cycle. This section discusses how external factors can influence them.

9.2.1 GROWTH

Chen et al. (2007) studied the feeding and growth in *A. japonicus* (wet weight range of 25.24–59.11 g, average of 36.89 g) reared at a range of temperatures typical of spring and autumn in northern China (9, 12, 15, 18, 21, and 24 °C). They showed that body wet weight increased under all conditions but that 15 °C was the optimum temperature (Figure 9.18). Furthermore, aestivation in *A. japonicus* began when temperature was above 22 °C, and ceased as soon as the temperature dropped below 20 °C. Finally, *A. japonicus* stopped feeding at 24 °C (Figure 9.19) (see Chapter 11 for details on aestivation in *A. japonicus*).

In another experiment, Chen et al. (2007) reared *A. japonicus* under various salinities (23, 26, 29, 32, 35, and 38) at the optimal temperature of 15 °C. Growth was recorded at salinities between 29 and 33 (Figure 9.20), but was maximum at 32 and minimum at 23 (Figure 9.21).

9.2.2 SPAWNING PATTERNS

The spawning behavior of adult *A. japonicus* is related to the phase of the moon. Taha et al. (2013) studied the spawning behavior in red and green color variants of *A. japonicus* in Japan (see Chapter 3 for details on color variants). They found that green *A. japonicus* spawned from March to April, whereas the

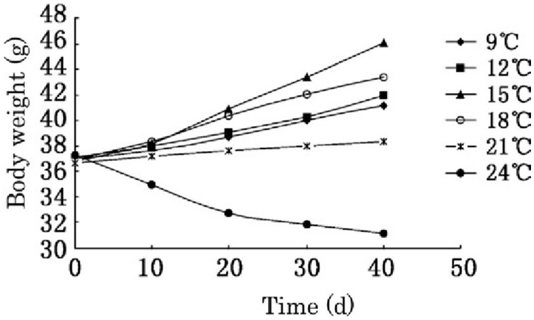

FIGURE 9.18

Weight increment in *A. japonicus* under various culture temperatures.

From Chen et al., 2007

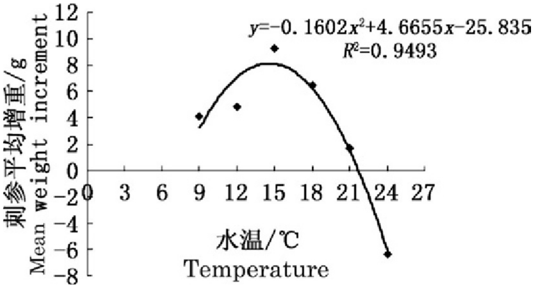

FIGURE 9.19

Relationship between temperature and sea cucumber mean weight at intervals of 40 days.

From Chen et al., 2007

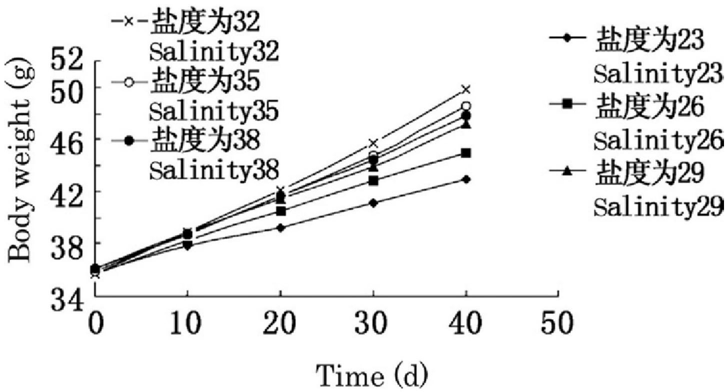

FIGURE 9.20

Weight increment in *A. japonicus* under different salinities.

From Chen et al., 2007

red variant spawned from April to May in Toyama. The green variant released most oocytes on bright nights (around the full moon). In contrast, the red variant showed no correlation with full moon nights and instead released gametes on dark nights between April 15 and May 13 (Table 9.2) (Taha et al., 2013).

9.2.3 AESTIVATION

A. japonicus is a temperate species that exhibits a period of low metabolic activity known as aestivation (see Chapter 11 for details). The most noticeable features of the aestivation period are cessation of feeding, weakening of activity patterns, gastrointestinal degradation, depressed metabolic activity, and body weight loss. When the water temperature rises to a threshold level (Table 9.3, Dong and Dong, 2009), sea cucumbers gradually stop feeding. During aestivation, they exhibit little movement,

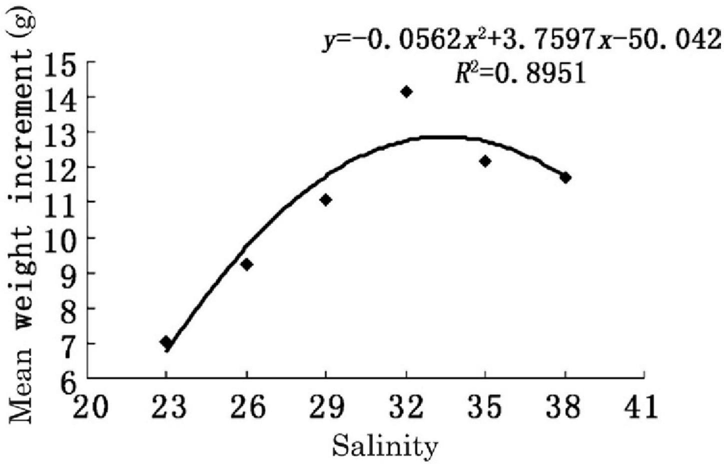

FIGURE 9.21

Relationship between salinity and mean weight increment of *A. japonicus* over 40 days).

From Chen et al., 2007

Table 9.2 Details of Oocyte Released ($\times10^6$) by Green and Red Color Variants of *A. japonicus*

		Green Variants		
Lunar Phases	**Breeding Date**	**Tank 1**	**Tank 2**	**Red Variants**
	March 1	32.00	0.00	0.00
	March 5	0.00	28.00	0.00
	March 6	20.60	0.00	0.00
	March 13	0.00	10.00	0.00
	March 15	0.00	14.00	0.00

(Continued)

Table 9.2 Details of Oocyte Released ($\times 10^6$) by Green and Red Color Variants of *A. japonicus* (cont.)

		Green Variants		
Lunar Phases	**Breeding Date**	**Tank 1**	**Tank 2**	**Red Variants**
	March 25	0.00	23.00	0.00
	March 29	22.30	0.00	0.00
	April 13	4.62	0.00	0.00
	April 15	0.00	0.00	3.80
	April 17	0.00	0.00	6.00
	April 30	0.00	0.07	0.00
	May 13	0.00	0.00	2.60
	Total	79.52	75.87	12.4

From Taha et al. 2013

and often remain still, while daily activities are reduced and most individuals are hiding under rocks or artificial substrata. Sheltering behavior during aestivation means that divers have a hard time finding them. This is therefore a more difficult period for surveying and harvesting sea cucumbers.

In China, *A. japonicus* enters aestivation later at higher latitudes; in mid- to late June in the southern coast of Shandong Province, in early or mid-July in northern coastal areas of Shandong, and in mid- to late August in the Liaodong Peninsula coast (Dalian area). However, aestivation ends roughly at the same time everywhere, usually between late October and early November. Therefore, aestivation lasts a minimum of two months and a maximum of nearly four months, depending on the region.

Table 9.3 Threshold Temperatures Enhancing Aestivation in the Sea Cucumber *A. japonicus* in Different Locations of its Geographic Distribution

Sites	Body Weight (g)	Threshold Temperature (°C)	References
Nanao Bay, Japan		20	Tokushisa, 1915
Miyagi, Aichi, Tokushima, Kagoshima, Hokkaido, Japan		19 ~ 22–18 ~ 23	
Dalian, Sea area, China		20 ~ 24.5	Ji et al., 2007
Dalian, Indoor, China	146.7 ± 5.5	24.5 ~ 25.5	Dong and Dong, 2006a
	84.0 ± 3.5	24.5 ~ 25.5	
	42.5 ± 2.1	24.5 ~ 25.5	
Penglai, Sea area, China	25 ~ 85	24.1	Liu et al., 1996
	86 ~ 160	22.9	
	> 160	21.8	
Qingdao, Sea area, China	Big size	20	Yu and Song, 1999
	Small size	25	
Qingdao, Indoor, China	37.3 ± 4.1	26	Ji et al., 2008

From Dong and Dong, 2009

The aestivation of *A. japonicus* is triggered by internal (metabolic) and external factors (food, light, temperature). Seawater temperature is the most direct and most significant factor of all. In the waters of northern China, it appears that the temperature that induces aestivation varies between 20.0 and 24.5 °C. Those differences could be related to the environments in which the sea cucumbers live or to different combinations of physical conditions.

9.2.4 HIBERNATION

Dong and Dong (2009) and Yu and Chang (2008) found that low temperature can also induce *A. japonicus* to exhibit reduced or null growth and lower metabolic activity. Hu et al. (2011) indicated that, when the water temperature is below 5 °C, the daily locomotory activities of *A. japonicus* gradually slow down; below 1 °C, sea cucumbers gradually stop moving, entering a state of hibernation. When the water temperature rises back to 4 °C, active feeding resumes.

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CHAPTER

FEEDING, DIGESTION, NUTRITIONAL PHYSIOLOGY, AND BIOENERGETICS

10

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SUMMARY

The sea cucumber *Apostichopus japonicus* is a deposit feeder. This chapter examines its feeding biology, focusing on feeding habits, selectivity, and seasonal variation in food sources, digestive enzymes, and nutritional requirements. The influence of feed composition, including protein sources, on growth rates and metabolic and immune responses are also discussed.

Keywords: *Apostichopus japonicus*; digestive enzymology; energy budget; feeding; food sources; nutritional requirement; sea cucumber

10.1 FEEDING HABITS AND FOOD SOURCES

10.1.1 FEEDING HABITS

The sea cucumber *Apostichopus japonicus* is a deposit feeder that finds food in two main ways: (1) on the surface of soft sediment, the oral tentacles spreading over the surface of the substrate and conveying the fine layer of organic material into the mouth; (2) on a rocky bottom, with the tentacles sweeping particles to the mouth (Figure 10.1). When feeding, the oral tentacles are extended, touching the substrate surface in a sweeping motion; the tentacles subsequently lift up and withdraw, then turn over inside the mouth. The food particles are offloaded in the pharynx, adhere to it, and are transferred into the digestive tract by the constriction of the pharynx (Zhao and Yang, 2010). See Chapter 4 for details on the general anatomy and Chapter 9 for diel and seasonal feeding periodicities.

10.1.2 DIET AND FEEDING SELECTIVITY

Feeding preferences of *A. japonicus* were mainly determined by analyzing gut contents, which revealed a mixture of various inorganic particles, fragments of marine plants, shell fragments, skeletal remains of echinoderms, debris, and other organic particles (Hauksson, 1979; Traer, 1980). Microscopic

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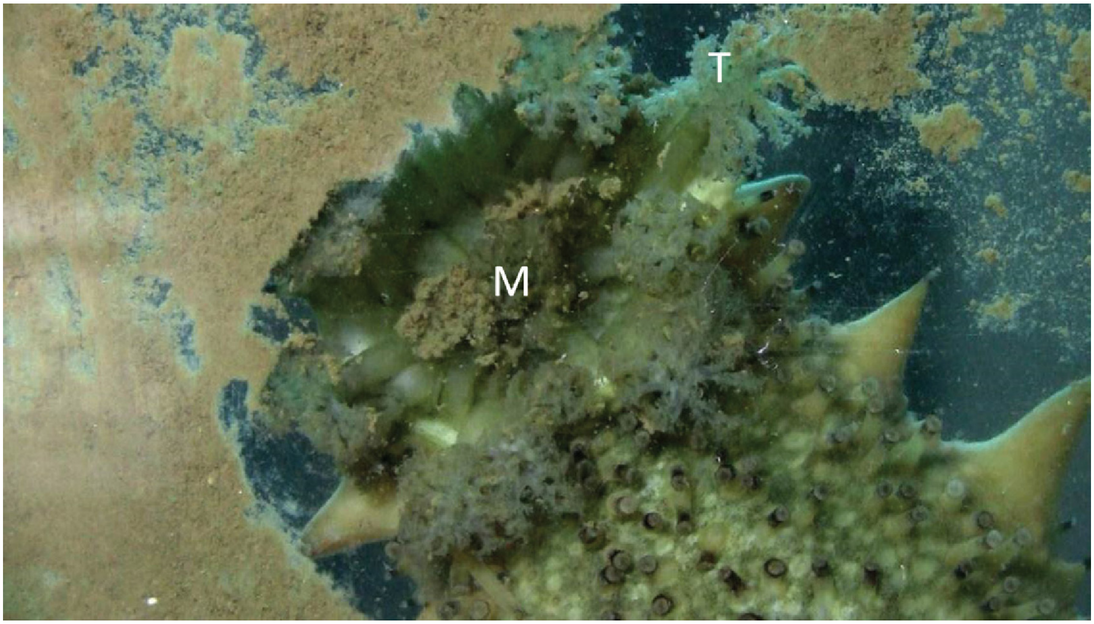

FIGURE 10.1

A feeding *A. japonicus*. Tentacles (T) surrounding the mouth (M) seen here through the bottom of a glass tank, are used to collect food particles.

Photo by Pan Yang

examination further revealed that there was a variety of small benthic organisms and microorganisms, such as bacteria, diatoms, and fungi. The gut content of *A. japonicus* in Hokkaido (Japan) mainly comprised particles of different sizes and shell pieces, including diatoms (60 species), algae fragments, protozoans (14 species), snail and bivalve larvae, copepods, shells of shrimps and crabs, eelgrass, wood chips, dust, and bacteria (Kinoshita and Tanaka, 1939).

Gut content of juveniles of *A. japonicus* was shown to be closely related with its body size. Small individuals weighing 2–2.5 g contained only a small quantity of sediment, being mostly composed of benthic diatoms and organic detritus. But with increasing size, the ratio of sediment in the gut content increased (Kinoshita and Tanaka, 1939; Sui, 1988).

Some researchers reported that sea cucumbers including *Holothuria mexicana*, *Isostichopus badionotus*, and *H. arenicola*, showed no preference for particle size (Hammond, 1982); indeed, large individuals of *I. badionotus* (Sloan and Vonbodungen, 1980), *H. atra* and *Stichopus variegatus* (see Hammond, 1982 for review) were suggested to be nonselective. However, other researchers argue that the organic content of food ingested by some deposit-feeding sea cucumbers is higher than that of sediment, indicating that deposit-feeding sea cucumbers may have chemical selectivity for food (reviewed by Zhao, 2010). Electron microscopy analysis showed that there are mucous vesicles with high electron density in the mastoid of the tentacle of *A. japonicus*. These vesicles are considered to be morphologically and histochemically similar to mucous vesicles found in other invertebrates (Hermans, 1983).

Bouland et al. (1982) proposed that the mastoid of *H. forskali* is homologous to the sensitive bumps of the spherical fork spines of sea urchin. The main function of these nonciliated cells on the papillary epithelium is touch (Bouland et al., 1982). The tentacle mastoid can not only secrete mucous to capture particles, but also receive and transmit external signals. Nonciliated cells can feel mechanical and chemical stimulation (Fankboner, 1978).

In a study of the suspension-feeding sea cucumber *Cucumaria pseudocurata* (Dendrochirotida), the papilla of the tentacles were found to consist of many mucilage cells covered with microvilli. These cells were found to contain mucous, which can glue the food to the mastoid (Hermans, 1983). There is also another type of ciliated mucous cell with a structure and function similar to that described by Burke (1980). Hermans (1983) believed that there was only one type of mucous cell on the surface of the tentacle of the sea cucumber *C. pseudocurata*, but that the tentacles could not offload their catch themselves. A study on *A. japonicus* showed that tentacles are withdrawn from the sediment surface and inserted in the oral cavity at which time the food particles are scraped off along the folds of the pharynx (Zhao et al., 2013). However, whether sea cucumbers have the ability to select or recognize sediment particles remains a matter of debate.

10.1.3 SELECTIVITY ON SEDIMENT GRAIN SIZE

Zhao and Yang (2010) reported that in muddy areas (sediment particle size $<63\,\mu m$) of Jiaozhou Bay (China), no difference was found between the size of particles in the gut contents of *A. japonicus* and grain size of the surrounding sediment. However, in the cofferdam and bottom culture areas of Qiansandao Island (China), particle selectivity by sea cucumbers appears to increase gradually as sediment particle size of the surrounding sediment becomes larger; the gut contents of *A. japonicus* had higher proportions of particles with a size between 1 and $80\,\mu m$ than the surrounding sediment. Alternatively, the result of field investigations may reflect particle availability rather than selectivity (Powell, 1977; Zhao and Yang, 2010).

10.1.4 FEEDING PREFERENCE AND INFLUENCE ON SURFACE SEDIMENTS

Deposit-feeding sea cucumbers need to continuously ingest large amounts of sediments, with generally low organic matter content, in order to meet their energetic requirements. The process of deposit feeding therefore reduces the organic matter content of the sediment surface. In turn, it supplies ammonium wastes to benthic microalgae and other microorganisms, thereby increasing their general abundance and diversity (Uthicke and Klumpp, 1998).

To test whether *A. japonicus* would exhibit any selectivity based on food type, feeding experiments were conducted in indoor tanks (Xia et al., 2012). Six experimental diets were prepared from powdered mixtures of sea mud and different species of plants/seaweeds (*Sargassum thunbergii*, *S. polycystum*, *Zostera marina*, *Ulva lactuca*, fresh *Laminaria japonica*, boiled *L. japonica*). A rare-earth oxide was incorporated as an inert marker (La_2O_3, Dy_2O_3, Eu_2O_3, Tb_2O_3, Sm_2O_3, Y_2O_3 for the respective diet treatments). The vegetal powder and mud were well mixed and offered in replicate patches of 4 cm diameter (see Figure 10.2 for experimental design). The model detailed

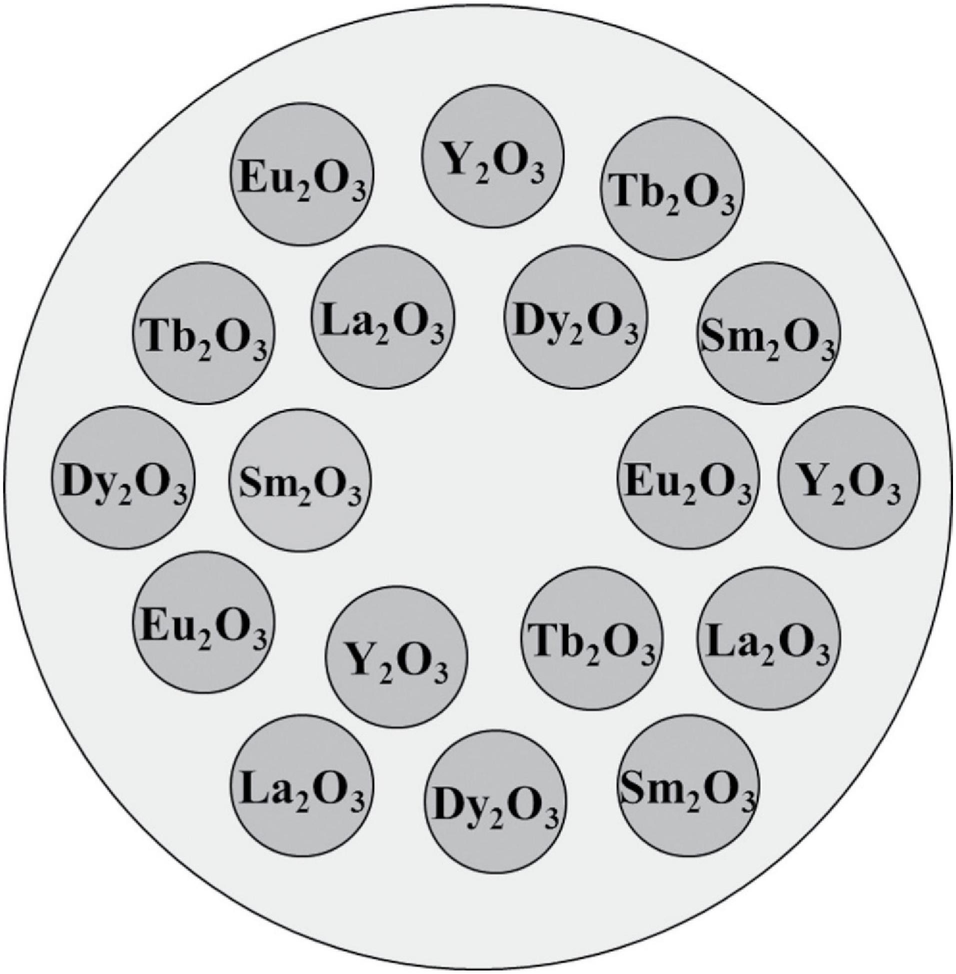

FIGURE 10.2

Food patches designed for diet selectivity experiment on *A. japonicus* with different organic and chlorophyll content. The diets and markers are as follows: La_2O_3 for *S. thunbergii*, Dy_2O_3 for *S. polycystum*, Eu_2O_3 for *Z. marina*, Tb_2O_3 for *U. lactuca*, Sm_2O_3 for fresh *L. japonica*, and Y_2O_3 for boiled *L. japonica.*

From Xia et al., 2012

by Xia et al. (2012) was used to assess food preference. Briefly, suppose a feed consists of n types of seaweed diets, and the ratio of the *L. japonica* seaweed in the diet is N_j. If the ratio of the *L. japonica* seaweed diet in the total diet ingested is A_j, then the food preference coefficient of the *L. japonica* seaweed diet γ_j can be defined as

$$\gamma_j = \frac{A_j}{N_j}$$

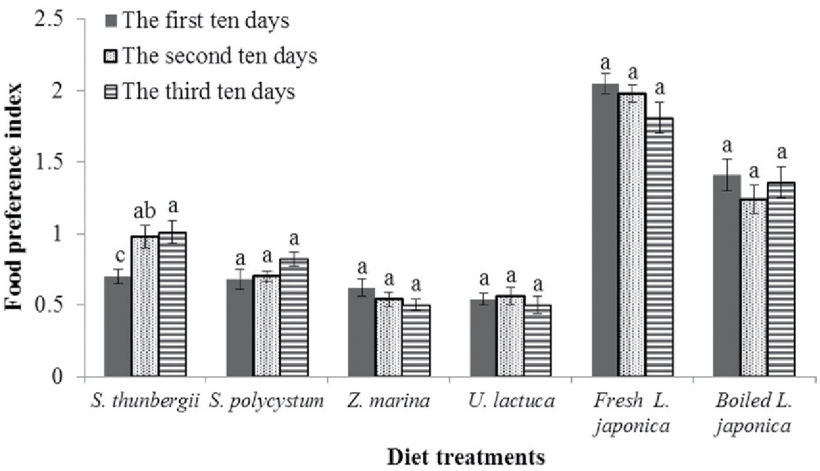

FIGURE 10.3

Feeding preference index of the sea cucumber *A. japonicus* presented with six different powdered diets. See text for definition of this index.

From Xia et al., 2012

A value of $\gamma_j > 1$ indicates food preference, $\gamma_j = 1$ indicates random choice, and $\gamma_j < 1$ indicates food aversion. Results showed that the food preference index of *A. japonicus* varied over time. The index for the *S. thunbergii* diet increased significantly over the 30 days of the experiment, shifting from aversion (0.70) in the first 10 days to preference (1.01) in the last 10 days ($P < 0.01$), as did the index for the *S. polycystum* diet. In contrast, the index for the fresh *L. japonica* and *Z. marina* diets declined with time, although there was no significant difference between the two (Figure 10.3).

Another laboratory study conducted by Michio et al. (2003) showed that *A. japonicus* could inhibit growth of algae and decrease the organic matter contents in a tank. Results showed that chlorophyll *a* concentration recorded in the surface sediment of the tank in the presence of *A. japonicus* (6.1 $\mu g\,g^{-1}$) was lower than that without them (60 $\mu g\,g^{-1}$). Phaeophytin concentration at the sediment surface in the presence of *A. japonicus* (0.9 $\mu g\,g^{-1}$) was also lower than that without them (4.5 $\mu g\,g^{-1}$). Feeding also reduced the total organic carbon (TOC) concentration at the surface of the sediment from 4.0 to 2.6 $\mu g\,g^{-1}$ (Michio et al., 2003).

10.1.5 COMPOSITION AND SEASONAL VARIATION IN FOOD SOURCES

The composition of food sources is very complex and closely related to the sources of organic matter in the sediment. Gao et al. (2010) studied the seasonal food sources of *A. japonicus* cultured in a cofferdam in Qingdao (China) using fatty acid biomarkers. Results showed that diatoms, dinoflagellates, protozoa, brown algae, green algae, and a variety of heterotrophic bacteria are potential food sources. Seasonal variation was also found. Overall, Gao et al. (2010) determined that:

- The high value of the diatom fatty acid signature suggests that diatom is a main component of the food sources (Figure 10.4A). Values were very high in winter and spring, but relatively low in summer and autumn. Diatoms often bloom in late winter and early spring.

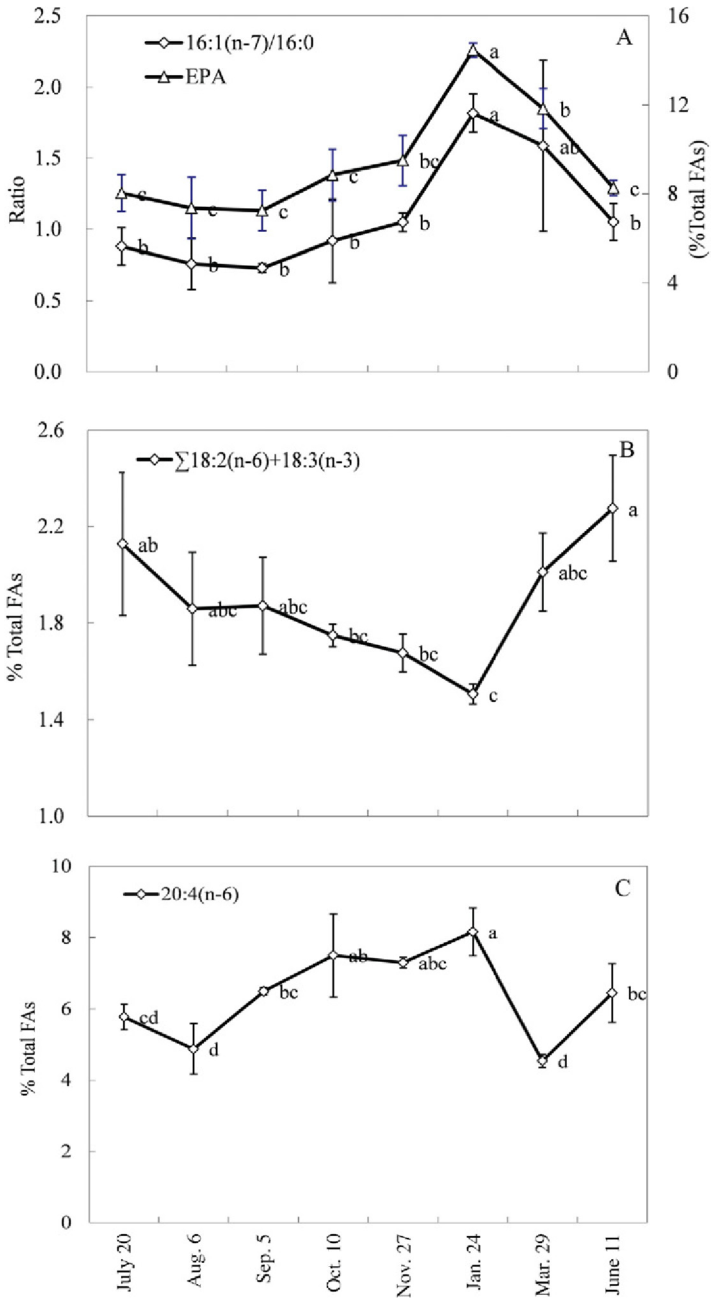

FIGURE 10.4

Seasonal variation of fatty acid biomarkers in the body wall of *A. japonicus*. (A) Diatom marker 16:1/16:0 ratio and 20:5(n-3) (EPA) content. (B) Chlorophyta marker Σ 18:2(n-6)+18:3(n-3) content. (C) Phaeophyta marker 20:4(n-6) content. Data shown as means with standard errors. Different letters above bars show significant differences between dates.

From Gao et al., 2010

- Brown macroalgae are another important food source for *A. japonicus* (Figure 10.4C). Its fatty acid biomarker 20:4n-6 was consistently high (4.9–8.2%) over the entire year, especially in autumn and winter. Brown algae are known to grow fast in winter and spring, and brown algae-derived debris supplied food sources to the sea cucumber in these seasons. In June, Chlorophyta in ponds begin to replace the brown algae and become the dominant group. As a result, the proportion of green algae-derived organic matter also increased in the diet of *A. japonicus* (Figure 10.4B). It is worth noting that the enrichment of 20:4n-6 is an important feature of echinoderms (Howell et al., 2003; Takagi et al., 1980). Besides widely accepted brown algae, the source of 20:4n-6 may also include some diatoms (Dunstan et al., 1994), protozoa and micro-eukaryotic cells (microeukaryotes) from the sediment, as well as free-living or endosymbiotic bacteria (Jøstensen and Landfald, 1997; Nichols et al., 1993; Russell and Nichols, 1999; Yano et al., 1997). Hence, the source of 20:4n-6 in the body wall of *A. japonicus* remains ambiguous and needs to be clarified.
- Odd and branched fatty acids (odd & br FAs) and 18:1n-7 are bacterial fatty acid biomarkers; the former mainly indicates Cytophaga-Flavobacteria and Gram-positive bacteria, the latter indicates Proteobacteria and Gram-negative bacteria. In summer and autumn, the level of odd & br FAs in *A. japonicus* is higher than in winter and spring. However, 18:1n-7 biomarker levels showed an opposite seasonal change. The minimum value appeared in September, while the maximum value appeared in January (Figure 10.5A). Results support that bacteria are important food sources for *A. japonicus*. The Cytophaga-Flavobacteria and Gram-positive bacteria fatty acid marker level remained high during the aestivation period from July to September, although the feeding rate was very low. The reasons may be as follows: (1) in summer, various types of organic matter (including brown algae) in the sediment faced severe decomposition mediated by Cytophaga-Flavobacteria, most of which are strictly aerobic or facultative anaerobic heterotrophic bacteria. They can produce a variety of extracellular hydrolytic enzymes, which can degrade agar, cellulose, and chitin, and thereby become the most important ecological link in the degradation process of complex organic substances (such as marine plants and crustaceans). During the degradation of organic matter, bacteria, together with organic detritus were ingested by sea cucumber as food source. (2) Meziane et al. (1997) studied several marine invertebrates (*Corophium volutator*, *Arenicola marina*, *Cerastoderma edule*) and found that, after being starved for a week, the bacterial fatty acid biomarker was still exhibiting a high level. Nereid worms (*Nereis diversicolor*) bacterial fatty acid marker level can be higher than the sediment sample collected in the field even after starvation. The author proposed that this may be because the amount of specific compounds that regulate the growth of gut bacterial community decreased markedly during starvation, which led to a bloom of bacteria (Meziane et al., 1997). This phenomenon might also exist in *A. japonicus* when it starves during aestivation (in summer).
- The flagellate protozoan fatty acid biomarker (DHA) remained at a low level with a maximum of only 4%, indicating that its importance may be lower than other food sources for *A. japonicus* (Figure 10.5B, C).
- The seasonal variation of different food sources was detected using principal component analysis on fatty acid biomarkers. In January, *A. japonicus* feeds mainly on diatoms, dinoflagellates/protozoa, brown algae, and bacteria (proteobacteria and Gram-negative bacteria). In March, it mainly feeds on diatoms, dinoflagellates/protozoa, and large green algae. Green algae-derived food sources occupy a larger proportion of the diet in June.

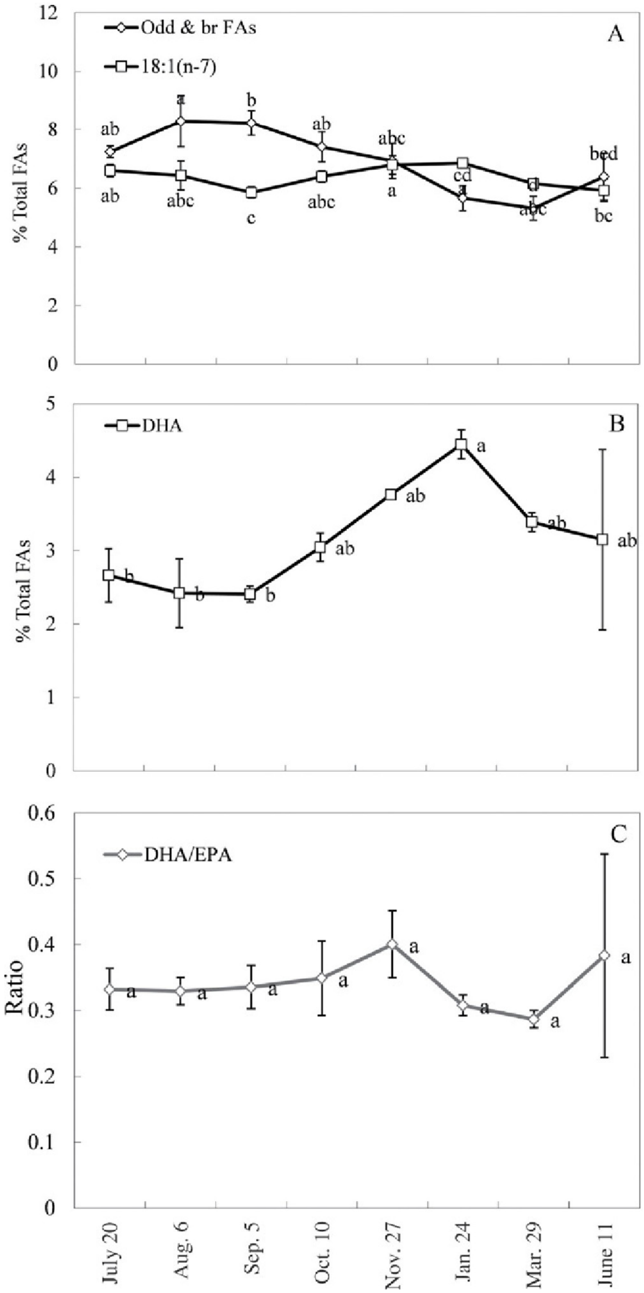

FIGURE 10.5

Seasonal variation of fatty acid biomarkers in the body wall tissue of *A. japonicus*. (A) Bacteria marker odd & br FAs and 18:1(n-7) content. (B) Flagellate/protozoan marker 22:6(n-3) (DHA) content and DHA/EPA ratio. Data shown as means with standard errors. Different letters above bars show significant differences between dates.

From Gao et al., 2010

In July, bacteria (Cytophaga-Flavobacterium class, Gram-positive bacteria) and large green algae accounted for a larger proportion of the food sources. Bacteria (Flavobacterium class, Gram-positive bacteria) accounted for a large proportion of food composition in August and September. Brown algae and bacteria (proteobacteria and Gram-negative bacteria) contributed a greater proportion to the diet in October and November.

10.2 DIGESTIVE ENZYMOLOGY

At the end of the nineteenth century and the beginning of the twentieth century, investigations in echinoderms confirmed the presence of several important enzymes in their digestive tract, leading to a surge of interest in the 1960s. These studies focused on the discovery of enzymes that can hydrolyze specific substrata, and on the evolution of these enzymes. However, few studies linked digestive enzyme activity to the nutritional demand of the animal (Jangoux and Lawrence, 1982).

Studies on the digestive enzymes of sea cucumbers were first carried out by Choe (1963), then Yokoe and Yasumasu (1964), Sova et al. (1970), and Favorov and Vaskovsky (1971). In recent years, with the rapid development of sea cucumber aquaculture in China, studies on the digestive enzymology of *A. japonicus* have grown in popularity.

10.2.1 CLASSIFICATION AND DISTRIBUTION OF DIGESTIVE ENZYMES

The sea cucumber *A. japonicus* possesses many types of digestive enzymes, including pepsin, trypsin, alkaline protease, serine protease (Fu, 2004), dipeptidyl peptidase, amylase, pectinase, cellulase, lichenase, and lipase (Choe, 1963).

Protease, lipase, and nonspecific esterase are located in the foregut and midgut epithelium (see Chapter 4 for details on anatomy); there are dense microvilli at the free end of midgut epithelial cells, and the free end of the plasma membrane has alkaline phosphatase activity. There are many subcutaneous sinuses with absorption function (Cui, 2000). The distribution of protease is complex in different segments of the digestive tract. Protease in the foregut is mainly in the form of pepsase and serine protease, with the highest activity occurring in the acidic (pH ~ 3.0) and the alkaline range (pH 7.0–9.0). Protease activity is low in the midgut, and almost nil in the acidic range of the hindgut (Fu et al., 2005).

During the larval development of *A. japonicus* (see Chapter 8), the type and concentration of digestive enzymes is more complex. The study of Tang et al. (2007), found that from auricularia to doliolaria larvae, protease activity decreased as the larvae lost feeding capability. It then increased again during the later stages of the development (pentactulae). Protease activity was higher in the juvenile than in the larva, and the amylase activity gradually increased to a maximum from auricularia to juvenile. During larval development, the activity of alginic acid degrading enzyme gradually increased and reached a peak in the pentactula, and then declined to its minimum value in the settled juvenile. Cellulase activity was very low during larval development and changed little, although it was enhanced at the juvenile stage (Tang et al., 2007).

Protease activity in the foregut and midgut of adult *A. japonicus* reached a maximum at 50 °C, while lipase and cellulase activity reached a maximum at 40 °C. Amylase activity in the foregut and midgut peaked at 40 and 30 °C, respectively (Jiang et al., 2007). Fu et al. (2005) studied the characterization of proteases from the digestive tract of *A. japonicus*. Results showed that acidic proteases exhibited optimum activity

at pH 2.0 and 5.0, and alkaline proteases at pH 8.0 and 13.5, respectively. Copper, Ca^{2+} and Mg^{2+} renatured the ethylenediamine tetraacetate (EDTA)-denatured protease at optimal pH ranges of 12–14. Jiang et al. (2007) found that under acidic conditions, protease activity in the foregut was higher than that in the midgut, but under alkaline conditions, the situation was inversed. Two relatively stable peak activity values of foregut lipase were found at pH 4.2–5.0 and 6.2–7.0. Maximum lipase activity in the midgut was found at pH 3.8, while lipase lost its activity at pH 9.0. Amylase activity in the foregut and midgut showed the same patterns with respect to pH variation. Amylase exhibited high and relatively stable activity at pH 6.6–7.4. Cellulase activity in the foregut and midgut were high at pH 6.2–7.4 and pH 5.4–7.0 (Jiang et al., 2007).

10.2.2 SEASONAL VARIATION OF DIGESTIVE ENZYME ACTIVITY

The activity of digestive enzymes exhibits seasonal variation in *A. japonicus* following changes in water temperature and food sources. Wang et al. (2007a) found that individuals from Dalian (northern China) showed an elevated activity for protease, amylase, and alginic acid-degrading enzymes with increasing water temperature from January to April–May. Thereafter, the activity gradually decreases as water temperature continued to rise. Enzyme activity exhibited a slight rebound from September to mid-October and declined to its minimum in December. Cellulose enzyme activity remained low and exhibited little seasonal change.

Gao et al. (2008) surveyed the annual fluctuation of amylase activity in *A. japonicus* and showed that it decreased from a maximum in July to a minimum in September and then generally increased again. The minimum amylase activity in September was significantly lower than the activity measured in June, July, August, and October (Figure 10.6A). The cellulase activity fluctuated over the year (Figure 10.6B). It decreased to a minimum ($0.02\,U\,mg^{-1}$ protein) in September and increased slightly thereafter (Gao et al., 2008).

In contrast to other carbohydrases, there was great variability in alginase activity within a given sampling date, and no differences were detected among sampling dates (Gao et al., 2008; Figure 10.6C). The activity remained constant from June to August, although it dropped to a minimum in September, as seen in the other carbohydrases. It remained at a high level from October to January. Pepsin activity also remained at a relatively stable level from June to August, but its activity increased to a peak in September (Gao et al., 2008; Figure 10.7A). Trypsin activity did not change over the duration of the study, but the highest values were detected in April (Gao et al., 2008; Figure 10.7B).

Lipase activity was found to change over the annual cycle (Figure 10.7C). The activity of this lipolytic enzyme exhibited a pattern similar to trypsin, peaking on 20 July at $6.50 \pm 0.90\,U\,mg^{-1}$ protein, and decreasing to a minimum activity of $1.51 \pm 0.70\,U\,mg^{-1}$ protein in October. The activity levels of lipase in October and November were lower than those observed in July (Gao et al., 2008).

The declining trends in digestive enzyme activity (amylase, alginase, cellulase) observed in *A. japonicus* during the aestivation phase (see Chapter 11) are consistent with data from lobster and shrimp, either in aestivation or under food deprivation (Comoglio et al., 2004; Johnston et al., 2004). Amylase (Sellos and Van Wormhoudt, 2002) and cellulase (Lo et al., 2003; Watanabe and Tokuda, 2001) are known to be of endogenous origin in invertebrates. Thus, the decrease in these endogenous enzymatic activities during a time of food deprivation (in the absence of substrata for the enzymes) makes sense (Caviedes-Vidal et al., 2000; Karasov, 1992). Alginase is known only from microbial

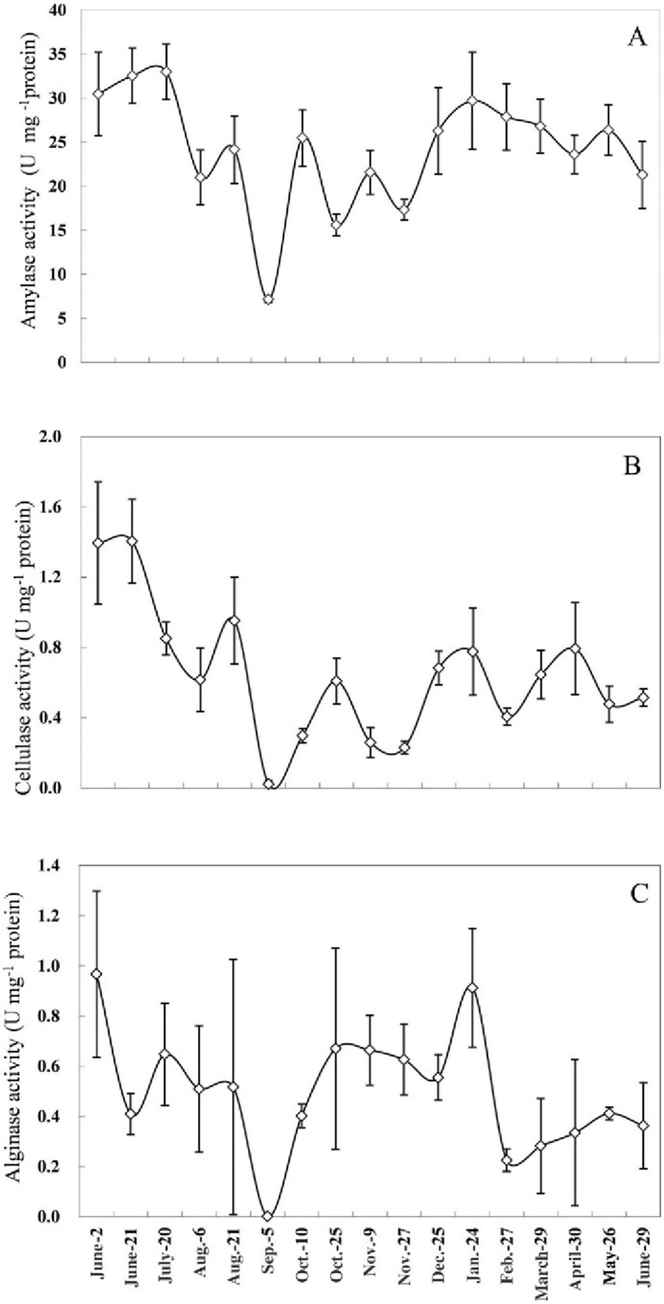

FIGURE 10.6

Seasonal variation in (A) amylase, (B) cellulase, and (C) alginase activity in *A. japonicus*. Values are expressed as means ± standard errors.

From Gao et al., 2008

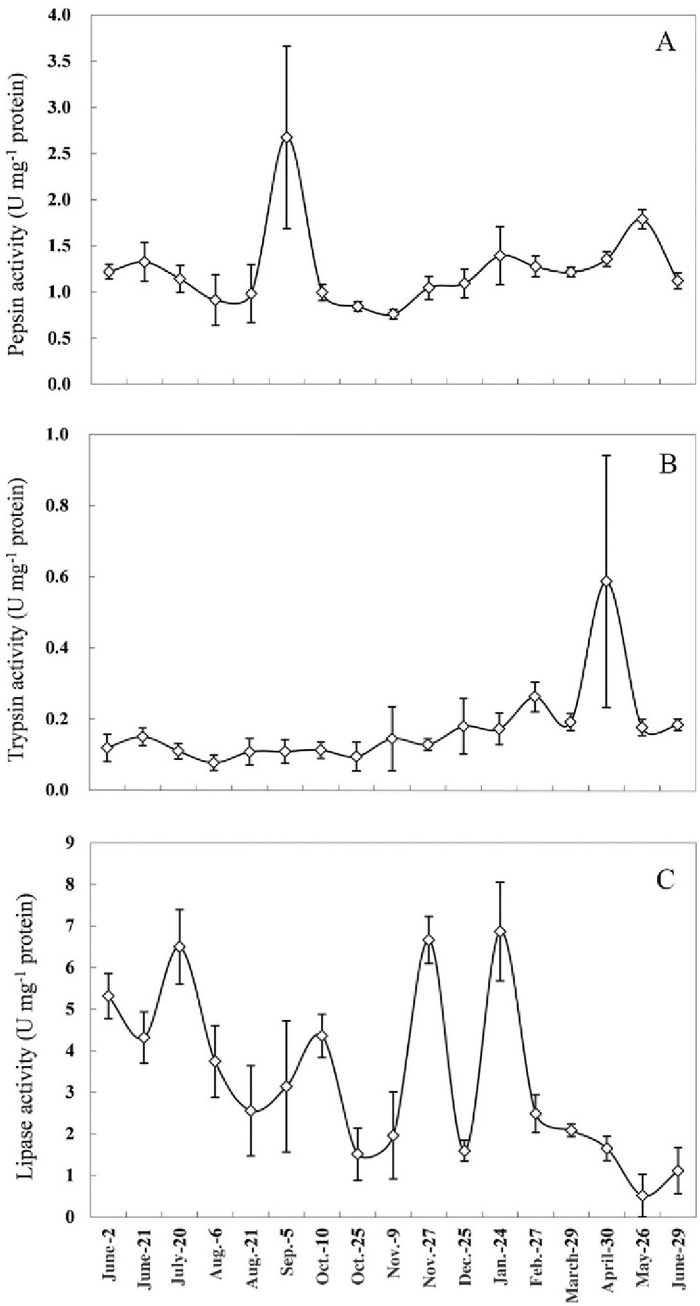

FIGURE 10.7

Seasonal variation in (A) pepsin, (B) trypsin, and (C) lipase activity in *A. japonicus*. Values are expressed as means ± standard errors.

From Gao et al., 2008

sources in the digestive tracts of invertebrates (Monje and Viana, 1998) and fishes (Skea et al., 2005, 2007), which may help explain the seasonally variable activity of the enzyme. Quite different from the other enzymes, pepsin activity suddenly spiked during the period of inactivity (September), to a much higher level than during the active phase (Gao et al., 2008). Moreover, an increase in pepsin activity was observed when *A. japonicus* was cultured at 28 °C, during which time it gradually ceased feeding and entered the inactivity phase (Gao et al., 2009). Fu et al. (2006) also found that pepsin activity in *A. japonicus* increased noticeably from June to July, with the inactivity phase in their study occurring between July and August. Gao et al. (2008) proposed that the increase in pepsin activity during food deprivation may simply reflect an abundance of pepsinogen stored in stomach tissues. The overall decrease in gut mass (Figure 10.8) would then result in an increase in pepsin activity per mg protein of homogenized gastric tissue. But if this is the case, the patterns of all enzymes should have been the same. Instead, only pepsin activity clearly spiked during the peak period of inactivity, and the reasons for this remain unclear.

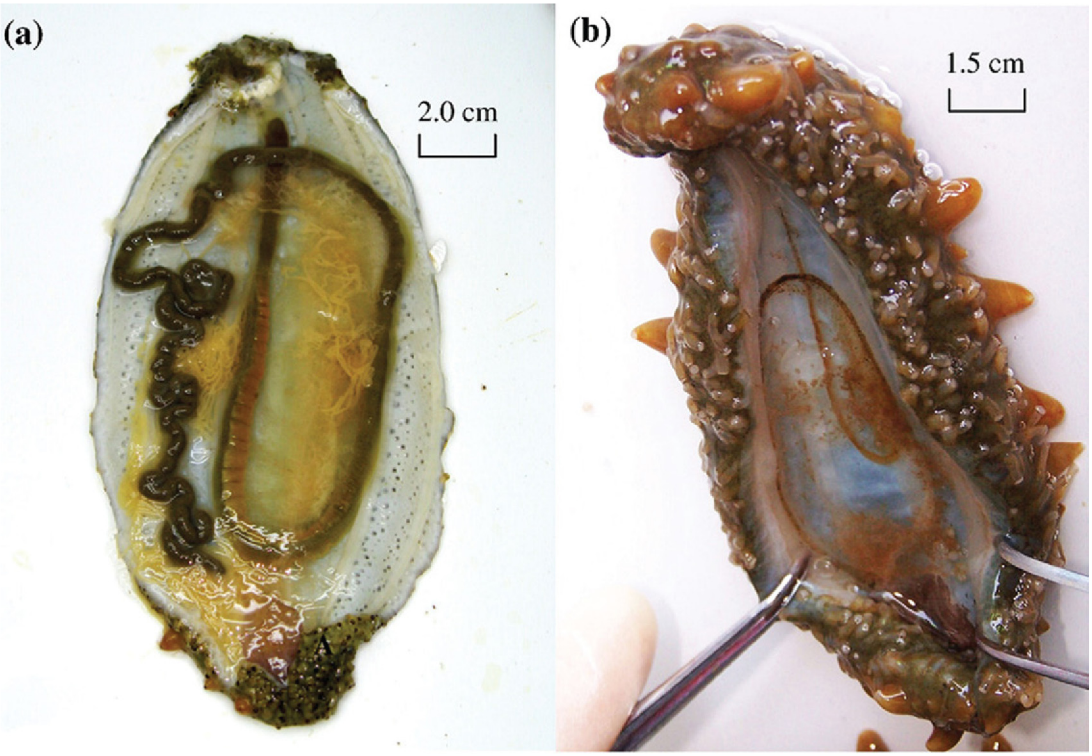

FIGURE 10.8

A comparison of the internal anatomy of *A. japonicus*. The digestive tract and respiratory tree (A) in active phase and (B) in the aestivation phase of the annual cycle, highlighting the atrophy of the digestive tract in B.

Photo by Gao Fei, from Gao et al., 2008

10.2.3 ADAPTIVE CHANGES IN DIGESTIVE ENZYMES

Guo et al. (2011) found that *A. japonicus* can regulate the function of the digestive tract and digestive enzyme activity in order to adapt to different sources of food. It allows the maintenance of high digestive enzyme activity in response to complex and varied food sources. When feeding on macroalgae-derived diets, such as *Enteromorpha*, *A. japonicus* adapts to develop digestive mechanisms similar to herbivorous animals by increasing the length of its digestive tract and secreting corresponding digestive enzymes to maximize digestion and utilization of food. The adaptation of enzyme activity can improve the animal's ability to acquire nutrition and energy from the environment (del Valle et al., 2004; Hassett and Landry, 1990).

When deprived of food, *A. japonicus* can initially acquire adequate intake of nutrients by increasing digestive enzyme activity. If the nutrient limitation period is prolonged, the physiological function of *A. japonicus* is inhibited, and thus the activity of digestive enzymes is reduced. Under nutrient limitation condition, crude protein and crude fat contents in the sea cucumber body was clearly lower than that of other food groups, so the drop in digestive enzyme activity may also be a manifestation of a decline in physical condition (Guo et al., 2011).

10.3 NUTRITIONAL PHYSIOLOGY

The sea cucumber *A. japonicus* mainly feeds on mud and detritus with low animal-based organic matter content, yet its nutritional need is not lower than that of other aquatic animals.

10.3.1 NUTRITIONAL REQUIREMENTS

Zhu et al. (2005) studied the protein and fat requirements of the sea cucumber *A. japonicus* using experimental feed consisting of powdered algae, white fish meal, and casein. Results showed that the most appropriate levels of crude protein and fat for juvenile food were 18.2–24.2% and 5%, respectively. Wang et al. (2009a) found that maximum specific growth rates were recorded in juvenile and adult sea cucumbers when the protein levels of prepared feed (made with fish powder, soybean powder, and seaweed powder) were 28.8 and 35.5%, respectively (with 3% fat content). Wang et al. (2009b) fed *A. japonicus* with fish meal and corn gluten as protein sources and fish oil as lipid source. The crude protein content of the feed was 19.8%. The fat requirement study results showed that the suitable fat fraction of the feed was 5.4–7.1% with an optimum of 5.9%. When the ratio of linoleic acid, linolenic acid, docosahexaenoic acid, and eicosapentaenoic acid content in the food was 10:5:6:1, sea cucumbers exhibited the fastest growth. Under these conditions, amylase and protease activity was highest in the intestine (Wang et al., 2009b). In addition, Li et al. (2009) showed that the phospholipid demand of *A. japonicus* was 0.7%.

Wang et al. (2007b) also studied the growth rate of *A. japonicus* fed with different protein sources. They showed that sea cucumbers can make good use of amino acids derived from plant protein sources, such as corn gluten and soy protein, which can also elevate the nutritional value of sea cucumber.

Wang et al. (2009c) further determined that dietary source of vitamin C could improve the growth of juvenile *A. japonicus*. The young sea cucumbers showed the highest apparent digestibility of dietary protein, the fastest specific growth rate, and the lowest feed conversion ratio when the feed contained 1000–1500 $mg\,kg^{-1}$ L-ascorbyl-2-polyphoshate (APP), 2000–2500 $mg\,kg^{-1}$ L-ascorbyl-2-monophosphate-magnesium (AMP), and 2000–3125 $mg\,kg^{-1}$ L-ascorbyl palmitate (AP). However, Okorie et al. (2008) proposed that the best feed contained AMP levels between 100 and

105.3 mg kg^{-1}. Furthermore, the weight gain, specific growth rate, and feed efficiency were higher in sea cucumbers fed with 120, 240, and 1200 mg ascorbic acid kg^{-1} of food in the form of AMP than with any other concentration tested. The contrasting results might partly be due to the size of the juveniles tested and the different feed recipes. Wang et al. (2009c) fed juveniles weighing ~2.3 g with corn protein and seaweed (*Sargassum* sp.) as protein and carbon sources, mixed with sea mud, whereas Okorie et al. (2008) fed juveniles of ~1.5 g with casein and wheat flour, mixed with oil and fish meal instead of sea mud.

10.3.2 EFFECT OF DIETARY PROTEIN

Xia (2012) showed that the activity of protease in the digestive tract of *A. japonicus* increased initially and then decreased with elevating dietary protein levels (Table 10.1). Protease, amylase, and lipase activity reached maximum values of 9.30, 34.0, and 15.7 U mg^{-1} protein, respectively, when dietary protein levels were between 10 and 20%. When the diet contained 5% protein, superoxide dismutase activity and total antioxidant index in the coelomic fluid showed the highest levels (Table 10.2). With 20% protein, lysozyme activity in the coelomic fluid showed the highest level.

Table 10.1 Effect of Dietary Protein Levels on Digestive Enzyme Activity (U mg^{-1} Protein) in the Sea Cucumber *A. japonicus* (Mean ± SD; n=3)

Index (U mg^{-1} Protein)	CP5	CP10	CP15	CP20	CP25	CP30
Protease	3.76 ± 0.54	6.97 ± 0.20	7.79 ± 0.54	9.30 ± 0.59	6.79 ± 0.47	6.88 ± 0.56
Amylase	33.09 ± 3.36	34.05 ± 6.58	30.63 ± 6.68	30.23 ± 9.49	31.50 ± 4.62	32.78 ± 1.63
Lipase	13.67 ± 2.89	12.33 ± 2.52	15.65 ± 6.25	14.30 ± 8.40	17.03 ± 16.86	15.56 ± 4.44

Diet treatments CP5, CP10, CP15, CP20, CP25, CP30 correspond to protein content (5, 10, 15, 20, 25, 30%, respectively).
From Xia, 2012

Table 10.2 Effect of Dietary Protein Levels on Various Immune Indices in the Sea Cucumber *A. japonicus* (Mean ± SD; n=3)

Index	CP5	CP10	CP15	CP20	CP25	CP30
TP (g L^{-1})	0.13 ± 0.02	0.13 ± 0.04	0.12 ± 0.02	0.13 ± 0.03	0.14 ± 0.02	0.14 ± 0.02
SOD (U mL^{-1})	53.99 ± 1.39	49.14 ± 5.43	51.64 ± 1.49	49.44 ± 9.88	47.02 ± 1.99	48.31 ± 3.38
LMZ (μg mL^{-1})	23.53 ± 6.79	24.84 ± 4.53	39.22 ± 3.92	36.60 ± 9.06	31.37 ± 7.84	32.67 ± 9.87
AKP (King U 100 mL^{-1})	0.63 ± 0.19	0.54 ± 0.21	0.46 ± 0.04	0.47 ± 0.23	0.45 ± 0.19	0.42 ± 0.17
T-AOC (U mL^{-1})	1.01 ± 0.13	0.92 ± 0.34	0.64 ± 0.09	0.97 ± 0.13	0.95 ± 0.13	0.98 ± 0.13
CAT (U mL^{-1})	0.26 ± 0.06	0.25 ± 0.6	0.23 ± 0.09	0.43 ± 0.31	0.23 ± 0.10	0.32 ± 0.15
MDA (nmol mL^{-1})	0.59 ± 0.12	0.39 ± 0.01	0.44 ± 0.12	0.68 ± 0.13	0.44 ± 0.03	0.51 ± 0.04

Diet treatments CP5, CP10, CP15, CP20, CP25, CP30 correspond to protein content (5, 10, 15, 20, 25, 30%, respectively).
TP: coelomic fluid protein; SOD: superoxide dismutase; LMZ: lysozyme; AKP: alkaline phosphatase; T-AOC: total antioxidant capacity; CAT: catalase; MDA: malonaldehyde.
From Xia, 2012

Xia (2012) also found that the rate of ammonia excretion first increased and then decreased with elevating dietary protein level (Figure 10.9). A diet with 25% protein elicited the highest ammonia excretion rate. The activity of glutamic-pyruvic transaminase (GPT) and gamma-glutamyl transpeptidase (GGT) in the coelomic fluid had a tendency to increase with the level of dietary protein (Table 10.3) (Xia, 2012).

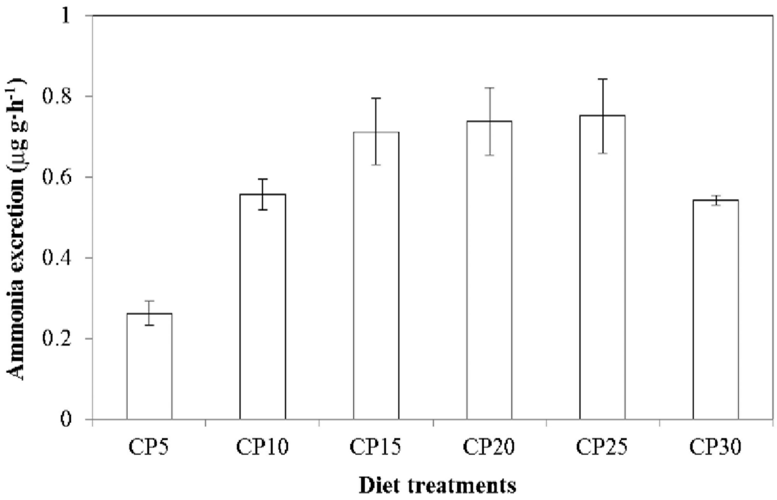

FIGURE 10.9

Effect of dietary protein levels on ammonia excretion in the sea cucumber *A. japonicus*. Diet treatments CP5, CP10, CP15, CP20, CP25, CP30 refer to protein content (5, 10, 15, 20, 25, 30%, respectively). Data shown as means and standard errors.

From Xia, 2012

Table 10.3 Effect of Dietary Protein Levels on Various Metabolic Indices in the Sea Cucumber *A. japonicus* (Mean ± SD; n = 3)

Index	CP5	CP10	CP15	CP20	CP25	CP30
BA (μmol L^{-1})	86.60 ± 20.91	64.17 ± 13.28	90.37 ± 18.54	91.09 ± 17.86	83.53 ± 22.90	104.10 ± 21.76
GPT (U L^{-1})	0.66 ± 0.25	0.80 ± 0.09	0.78 ± 0.45	0.81 ± 0.25	1.17 ± 0.47	1.10 ± 0.48
GGT (U L^{-1})	1.00 ± 0.04	0.94 ± 0.04	1.03 ± 0.14	0.99 ± 0.07	1.01 ± 0.08	1.31 ± 0.07

Diet treatments CP5, CP10, CP15, CP20, CP25, CP30 correspond to protein content (5, 10,15, 20, 25, 30%, respectively).
BA: ammonia content in coelomic fluid; GPT: glutamic-pyruvic transaminase; GGT: gamma-glutamyl transpeptidase.
From Xia, 2012

10.4 ENERGY BUDGET

The ingestion rate (IR) and absorption rate (AR) are key parameters affecting the energy budget of *A. japonicus*, and they can be very different depending on body size and food sources. Zhao and Zhang (2004) investigated IR and AR of deposited organic matter by *A. japonicus* of various sizes, and found that sea cucumbers with body weights of ~5.7, 13.8, and 58.8 g, at water temperatures of 13.2–22.3 °C, had average IRs of 3.24, 6.48, and 9.59 mg g^{-1} h^{-1} respectively, and average ARs of 7, 13.5, and 17.6%, respectively. At a water temperature of 15.1 °C, sea cucumbers with a body weight of ~58.8 g, exhibited the highest ARs (15.3%), while at a water temperature of 23.2 °C, the ones with body weight of ~5.7 and 13.8 g, exhibited the highest ARs (23.6 and 26.5%, respectively).

Yang et al. (2005) found that the maximum rate of food consumption in terms of energy increased following a power equation relationship as body weight of *A. japonicus* increased, whereas specific growth rates (SGRs) decreased. These trends were evidenced at temperatures of 10, 15, and 20 °C, but were not apparent at 25 and 30 °C, when SGRs were negative, due to aestivation.

Yuan et al. (2006) quantified the growth of *A. japonicus* in order to determine the proper diet for land-based intensive culture of this species. Individuals with an initial wet body weight of 32.5 ± 1.0 g were fed with five different granule diets containing dried bivalve feces and/or powdered algae. The five diets used in the experiment were: diet A – dried feces of bivalve; diet B – 75% dried feces and 25% powdered algae; diet C – 50% dried feces and 50% powdered algae; diet D – 25% dried feces and 75% powdered algae; diet E – powdered algae. Results showed that diets affected ingestion rates, feces production, food conversion efficiency, and apparent digestive ratios (Figures 10.10 and 10.11), hence the energy budget (Tables 10.4 and 10.5).

Table 10.4 Effect of Different Diets on the Energy Budget of *A. japonicus*

Energy Parameters	Diet treatments				
	A	B	C	D	E
C (J g^{-1} d^{-1})	215.1 ± 15.9^{a}	3133.6 ± 96.3^{b}	3833.5 ± 341.2^{b}	5122.3 ± 698.7^{c}	6676.2 ± 36.5^{d}
F (J g^{-1} d^{-1})	206.2 ± 53.3^{a}	1888.8 ± 187.4^{b}	2135.3 ± 203.1^{bc}	2932.2 ± 417.0^{c}	4479.9 ± 197.5^{d}
U (J g^{-1} d^{-1})	70.3 ± 12.6^{a}	84.5 ± 5.3^{ab}	93.0 ± 7.0^{ab}	121.5 ± 15.4^{b}	181.3 ± 12.5^{c}
R (J g^{-1} d^{-1})	108.2 ± 20.8^{a}	933.8 ± 135.5^{b}	1360.1 ± 136.0^{bc}	1669.9 ± 258.6^{c}	1664.3 ± 184.8^{c}
G (J g^{-1} d^{-1})	-169.7 ± 29.2^{a}	226.5 ± 34.12^{b}	245.2 ± 18.1^{b}	398.7 ± 44.4^{c}	370.7 ± 17.8^{c}
Ab (J g^{-1} d^{-1})	8.9 ± 37.4^{a}	1244.8 ± 100.5^{b}	1698.2 ± 148.7^{bc}	2190.1 ± 302.9^{c}	2196.3 ± 190.6^{d}
As (J g^{-1} d^{-1})	-64.5 ± 50.0^{a}	1160.4 ± 104.8^{b}	1605.2 ± 141.7^{bc}	2068.6 ± 287.9^{c}	2015.0 ± 192.3^{c}
K_1 (%)	-78.31 ± 7.80^{a}	7.18 ± 0.89^{b}	6.44 ± 0.43^{b}	7.85 ± 0.47^{b}	5.55 ± 0.24^{b}
K_2 (%)	-401.77 ± 169.26^{a}	18.78 ± 3.81^{b}	14.57 ± 1.28^{b}	18.46 ± 1.83^{b}	17.06 ± 1.28^{b}
G/R (%)	-168.22 ± 59.36^{a}	26.07 ± 6.38^{b}	18.29 ± 1.92^{b}	24.46 ± 3.14^{b}	22.98 ± 2.16^{b}

Diet treatments: A, dried feces of bivalve; B, 75% dried feces and 25% powdered algae; C, 50% dried feces and 50% powdered algae; D, 25% dried feces and 75% powdered algae; E, powdered algae.
C*: Energy intake;* F*: Energy lost in feces;* U*: Energy lost in excretion;* G*: Energy deposited as growth;* R*, Energy lost in respiration;* Ab*: Absorbed energy* (Ab = P + R + U = C − F)*;* As*: assimilated energy* (As = P + R = C − F − U = Ab − U)*;* K_1 *and* K_2*: Gross and net growth efficiency, respectively;* G/R*: Proportion between energy channeled into growth and respiration. Values (mean ± SE,* n = 3*) with different letters in the same line were significantly different from each other* ($p < 0.05$)*.*
From Yuan et al., 2006

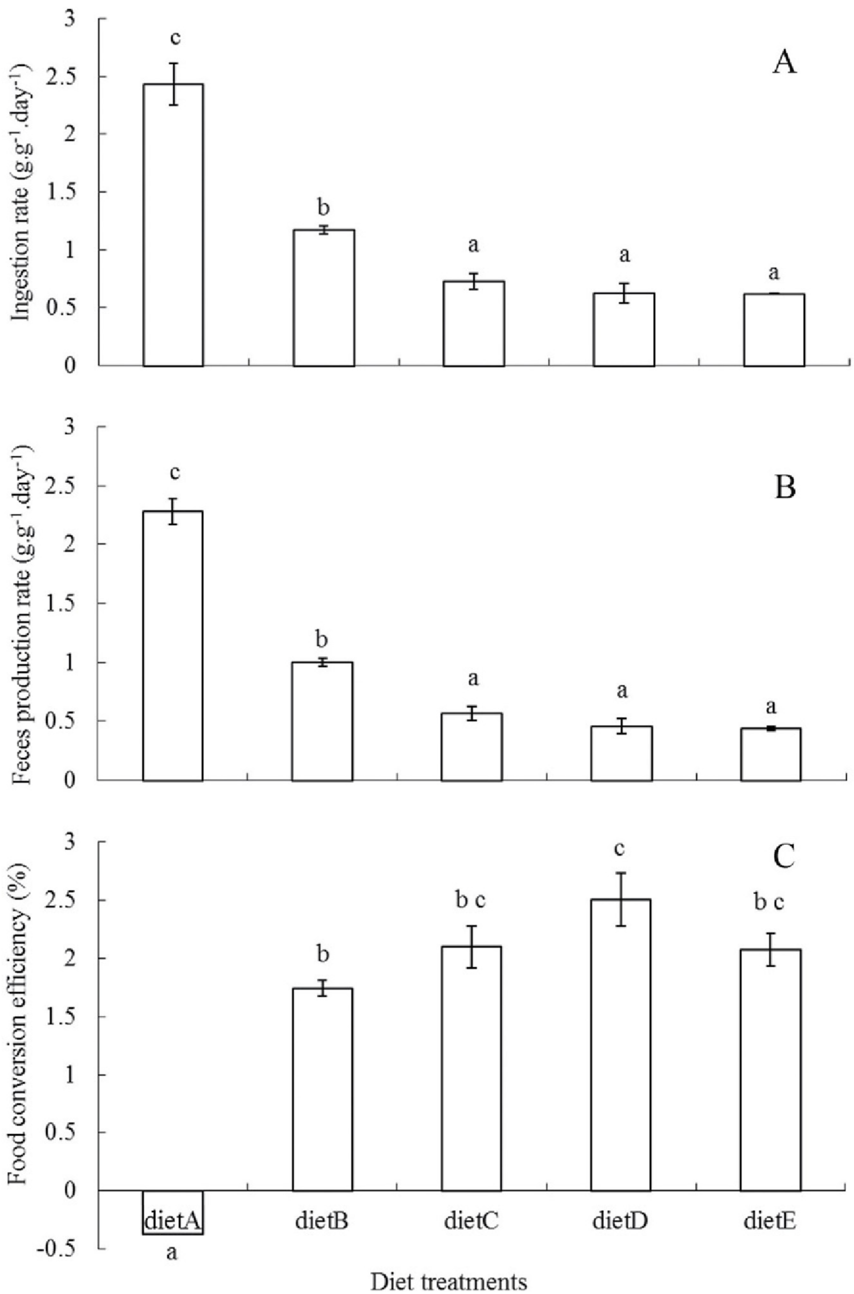

FIGURE 10.10

(A) Ingestion rate, (B) feces production rate, and (C) food conversion efficiency of *A. japonicus* fed different diets. Means (±SE, $n = 3$) with different letters denote significant differences ($p < 0.05$). Diets are defined in Table 10.4.

From Yuan et al., 2006

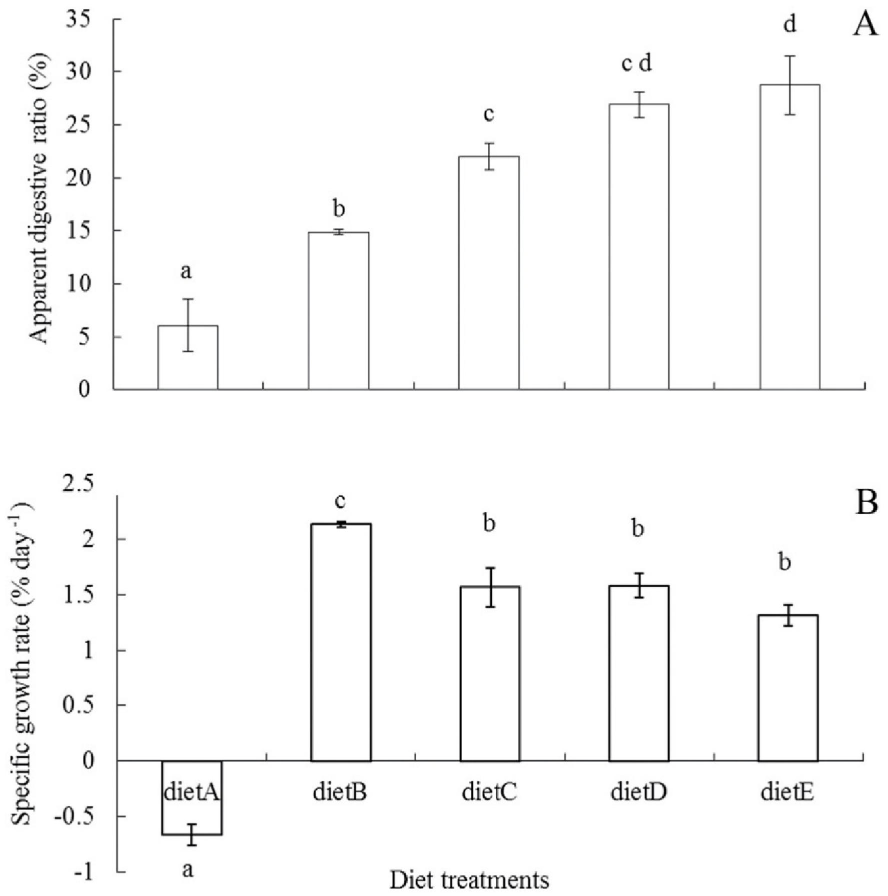

FIGURE 10.11

(A) Apparent digestive ratio and (B) specific growth rate of *A. japonicus* fed different diets. Means (±SE, $n = 3$) with different letters denote significant differences ($p < 0.05$). Diets are defined in Table 10.4.

From Yuan et al., 2006

The energy loss in feces for individuals of *A. japonicus* fed only with dried feces of bivalve was higher (206.2 J g^{-1} d^{-1}, accounting for 94.6% of the total energy consumed) than for those fed with dried bivalve feces mixed with different proportions of powdered algae. Sea cucumbers fed with diets mixed with different proportion of bivalve feces, however, showed a similar energy allocation. The energy deposited in growing tissues was 5.5–7.9%; energy lost in feces was between 55.6–67.1%, and energy lost in excretion and respiration was 2.4–2.7% and 24.6–35.5%, respectively. Yuan et al. (2006) stated that ingestion rates were negatively related to protein level or energy content. In natural ecosystems, sediment of low nutritional value need to be ingested in large quantities by deposit feeders in order to gain a net input of energy (Hudson et al., 2004; Santos et al., 1994). Inversely, when food quality increases, in certain seasons for example, appetite regulation would work actively to decrease food ingestion (Yuan, 2005). The same phenomenon was also reported in sea urchins (McBride et al., 1998; Otero-Villanueva et al., 2004).

Table 10.5 Patterns of Energy Allocation in *A. japonicus* Fed Different Diets

Diets	*C*	*G* (%C^{-1})	*F* (%C^{-1})	*U* (%C^{-1})	*R* (%C^{-1})
A	100	-78.31 ± 7.80^a	94.55 ± 17.80^b	32.45 ± 3.46^b	51.30 ± 13.47^b
B	100	7.18 ± 0.89^b	60.03 ± 4.40^a	2.69 ± 0.09^a	30.13 ± 5.31^a
C	100	6.44 ± 0.43^b	55.64 ± 1.19^a	2.43 ± 0.06^a	35.49 ± 1.44^{ab}
D	100	7.85 ± 0.47^b	57.14 ± 1.85^a	2.38 ± 0.04^a	32.63 ± 2.18^a
E	100	5.54 ± 0.24^b	67.10 ± 2.89^a	2.72 ± 0.20^a	24.63 ± 2.80^a

Diets are defined in Table 10.4.
C*: Energy intake;* F*: Energy lost in feces;* U*: Energy lost in excretion;* G*: Energy deposited as growth;* R*, energy lost in respiration.*
Values (mean ± SE, n = 3) with different letters in the same column were significantly different from each other ($p < 0.05$).
From Yuan et al., 2006

10.5 CONCLUSION

A. japonicus feeds on various particles including inorganic sediment and organic detritus. The composition of food sources is very complex and closely related to the sources of organic matter in the sediment. *A. japonicus* may exhibit selectivity on sediment grain size of 1–80 μm and prefer food patches with low organic and chlorophyll content. Diatoms, dinoflagellates, protozoa, brown algae, green algae, and a variety of heterotrophic bacteria are potential food sources. The diet composition also exhibits a remarkable seasonal variation. Several digestive enzymes have been found in *A. japonicus*, including pepsin, trypsin, super alkaline protease, serine protease, dipeptidyl peptidase, amylase, pectinase, cellulase, lichenase, and lipase. Due to differences in water temperature and food sources, the activity of digestive enzymes undergoes seasonal variation in individuals of *A. japonicus* living in different regions. *A. japonicus* can also regulate the function of the digestive tract and digestive enzyme activity in order to adapt to different sources of food. Dietary protein levels influence digestive enzyme activity, and immune and metabolic index. Ingestion and absorption rates are key parameters affecting the energy budget of *A. japonicus*, and they can vary markedly with body size and food sources.

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CHAPTER

AESTIVATION AND REGENERATION

11

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SUMMARY

The present chapter details and discusses the processes of aestivation and organ regeneration in *Apostichopus japonicus*. High seawater temperature in the summer triggers a dormant state, known as aestivation, in almost 100% of mature individuals. During this phase, *A. japonicus* undergoes major tissue loss, metabolic rate depression, and immune system modification. Morphological, molecular, and transcriptomic studies on aestivation in *A. japonicus* have helped define its main stages as well as the mechanisms of hypometabolism and gene expression silencing that underlie it. When *A. japonicus* is exposed to stressful conditions, it can shed most of its internal organs, which will later be regenerated. The process of intestine regeneration was investigated through light and electron microscopy analysis. The general underlying mechanisms mainly involve morphallaxis at the early stage and epimorphosis at the later stage. The gene expression profile during intestine regeneration in *A. japonicus* was constructed to provide significant information for future research on regenerative pathways.

Keywords: aestivation; *Apostichopus japonicus*; intestine; metabolism; molecular mechanism; regeneration; sea cucumber

11.1 AESTIVATION

As the water temperature rises in summer, *Apostichopus japonicus* undergoes aestivation, which is characterized by tissue degradation, hypometabolism, and immune system adjustment. A major decrease in body weight occurs, with serious impacts on production efficiency in aquaculture systems. Although the phenomenon of aestivation in sea cucumbers was first reported in 1897, in-depth research into it has only surged in recent years with studies in *A. japonicus* focusing on the regulation of this biological process.

11.1.1 DEFINITION OF AESTIVATION

Aestivation is generally defined as a type of dormancy, which is a survival strategy used to sustain lack of food and other extreme conditions (Pinder et al., 1992; Abe, 1995; Storey, 2002). Animals that aestivate become inactive and stop feeding in response to warm temperatures. Research on aestivation has focused on vertebrates, such as lung fish, amphibians, reptiles, small mammals, and certain

Wang, T., Sun, L., Chen, M., 2015. Chapter 11. Aestivation and regeneration. In: Yang, H., Hamel, J.-F., Mercier, A. (Eds.), The Sea Cucumber *Apostichopus japonicus.* History, Biology and Aquaculture, Academic Press, pp. 177–210. http://dx.doi.org/10.1016/B978-0-12-799953-1.00011-8

invertebrates, such as mollusks. The duration of aestivation varies among species; some enter this state for a few months, others for a longer period. During aestivation, the animals undergo important physiological and biochemical adjustments, such as changes in energy consumption, metabolic activity, and immune response. The molecular mechanisms underpinning these adjustments mainly include transcriptional and translational regulation, epigenetic modification, adenosine monophosphate (AMP), and Akt-mediated signal transduction (Akt is a cytosolic protein kinase also known as protein kinase B, PKB). Hypometabolism is considered key for the response to high temperature, drought, and other extreme conditions. The decrease in metabolic rate in aestivating animals, which can reach 70–80% of the resting value, and nearly 100% in some species, conserves energy to extend survival time (Pinder et al., 1992; Pedler et al., 1996; Guppy and Withers, 1999; Storey and Storey, 2010).

A. japonicus is a shallow-water temperate species and variation in water temperature is known to influence its growth (see also Chapters 8 and 9). When the seawater temperature rises to a certain level during summer, most individuals of *A. japonicus* migrate to deeper environments and stop moving and feeding, entering a dormant state dubbed aestivation, which lasts up to ~100 days. During this period, the sea cucumber experiences organ atrophy and major weight loss (Li et al., 1996), which has a serious impact on its production through aquaculture. Since sea cucumber aestivation was first reported in Japan over a century ago (Mitsukuri, 1897), theoretical models have been developed with research findings in ecology, morphology, physiology, molecular biology, and genomics.

Recently, the internal regulation mechanism of sea cucumber aestivation was investigated through a multifaceted approach, and basic theories on aestivation were improved. Studies have shown that aestivation in *A. japonicus* is characterized by the following: decreased metabolic rate (lower oxygen consumption rate and ammonia excretion rate), energetic adjustment (feeding and defecation are null and energy stored in the body is adjusted to maintain basic physiological activities, such as respiration and excretion), modified immune parameters (body cavity fluid cell decreased concentrations; activity of superoxide dismutase (SOD) and catalase (CAT) are adjusted), and degradation of intestinal structure and function (major gastrointestinal degradation and change in digestive enzyme activity). With the development of research techniques in molecular biology and bioinformatics, the molecular mechanisms underlying sea cucumber aestivation continue to be explored.

11.1.2 PRINCIPLES OF METABOLIC REGULATION IN AESTIVATING ANIMALS

Depression of metabolic rate has been recorded for major animal species in response to environmental stress; it was considered as a characteristic adjustment of aestivation. The decline of catabolism, protein synthesis, and ion channel activity are involved in metabolic regulation (Churchill and Storey, 1989; Storey and Storey, 1990; Guppy et al., 1994; Guppy and Withers, 1999). Terrestrial snails *Cepaea nemoralis* exhibited an 83.9% reduction in cytochrome-*e* oxidase activity in hepatopancreas during aestivation, which was associated with oxidative metabolism (Stuart et al., 1998). Similar regulation in catabolic depression has been found in land snails and marine molluscs (Churchill and Storey, 1989; Brooks and Storey, 1997). The suppression of protein synthesis was considered one of the first responses by most cells placed under stress or nutrient/energy limitation (DeGracia et al., 2002). The *in vitro* metabolic depression of liver in Australian desert frogs *Neobatrachus centralis* is accompanied by a 67% decrease in protein synthesis, which accounts for 52% of the metabolic depression of this tissue (Fuery et al., 1998). Ion channel activity depression contributed to the hypometabolism in aestivating animals; in the land snail *Otala lactea*, suppression of Na+/K+-ATPase and sarcoendoplasmic reticulum Ca2+-ATPase activity was found in tissues of foot muscle and hepatopancreas, and it was linked with the reversible phosphorylation

control of proteins (Ramnanan and Storey, 2006, 2008). Molecular research on the mechanism of hypometabolism has revealed that gene expression regulation, protein modification, and intracellular signaling cascades affect material and energy metabolism in aestivating animals (Storey and Storey, 2004, 2010).

11.1.3 DEGENERATION OF DIGESTIVE TRACT IN AESTIVATING *A. japonicus*

The digestive tract of sea cucumbers consists of six segments: the mouth, pharynx, esophagus, stomach, intestine (subdivided into anterior, middle, and posterior intestine), and cloaca (see Chapter 4 for details on anatomy). The wall structure of the intestine in *A. japonicus* is dominated by the mucosa, submucosa, muscularis, and tunica externa. Results from Cui et al. (2000) and Wang (2004) indicated that the gastrointestinal mucosa of sea cucumber is a single-layer or pseudostratified epithelium and is composed of columnar, cuboidal, and mucous cells. The submucosa is comprised of loose connective tissue. The muscle layer can be divided into inner longitudinal and outer annular layers. The tunica externa consists of flat cells and an underlying thin layer of reticular tissue. The organizational structure is slightly different according to each part of the digestive tract (Cui et al., 2000; Wang, 2004).

During aestivation, the intestine of *A. japonicus* undergoes marked morphological changes; it becomes very thin, with a diameter <1 mm and a length less than half that of the pre-aestivation intestine (Gao, 2008). During the aestivation period the ratio of digestive tract length to body length was found to decrease, whereas folds were reduced, columnar cells were shorter, villi and microvilli were almost completely lost, and secretory granules decreased in abundance or completely disappeared (Li et al., 2006; Wang et al., 2007). Gao (2008) found that sea cucumbers collected during aestivation had lighter digestive tracts and relative gut mass (RGM) representing only 8.2–35.8% of the annual maximum value (Figure 11.1). Moreover, the ZI (Zihler's index) of the digestive tract decreased to 28.0–60.4% of the annual maximum value (Figure 11.2) (Gao, 2008).

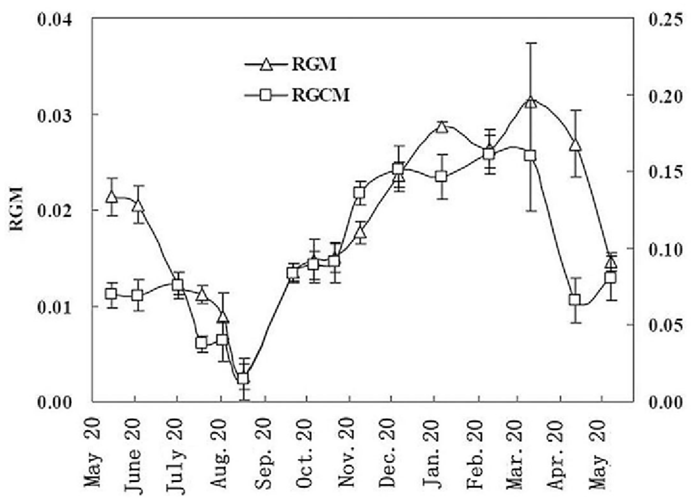

FIGURE 11.1

Seasonal variations of RGM (relative gut mass) and RGCM (relative gut content mass) in *A. japonicus*. Values are expressed as mean ± SEM.

From Gao, 2008

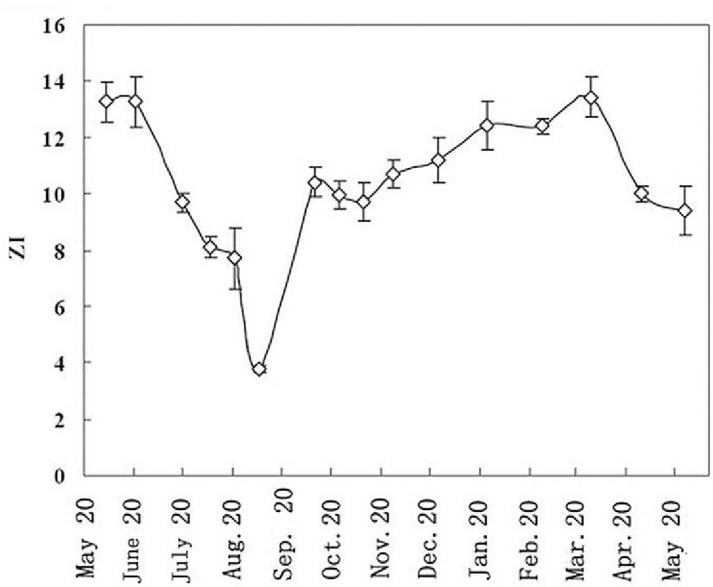

FIGURE 11.2

Seasonal variation of Zihler's Index (ZI) in *A. japonicus*. $ZI = GL/(10 \times BM^{1/3})$, where GL is gut length, and BM is body mass. Values are expressed as mean ± SEM.

From Gao, 2008

Wang et al. (2011) carried out histological observation and apoptosis detection of the digestive tract in *A. japonicus* induced into aestivation by temperature elevation. Results showed that the structure of the anterior intestine in the experimental group (25 °C; induced aestivation) changed dramatically. After five days, cavities developed as a result of apoptotic activity (H&E staining) and positive fluorescent signal points (TUNEL assay) were found in circular folds of the intestinal wall. After 10 days of temperature rise, the number and size of cavities and positive fluorescence signal points increased. After 20 days, the cavities disappeared and the intestinal wall became much thinner although it maintained structural integrity (Figures 11.3 and 11.4). Intestinal cell deletion by apoptosis in aestivating sea cucumber was suggested to contribute to the degradation of the digestive tract (Wang et al., 2011). Su et al. (2012) compared the intestinal epithelial ultrastructure between aestivating and nonaestivating sea cucumbers. In the arousal stage following aestivation (see definitions later), a series of changes occurred in the mucous epithelium, including a decrease in the number of microvilli, the condensation of nucleus chromatin, a decrease in mitochondria and vacuolization, a decline in the number of ribosome and a loss of pigments and other substances through cell lysis. However, nonaestivating sea cucumbers had more microvilli in the intestinal epithelial cells; chromatin mainly appeared in the form of autosome, the nucleolus was clear, and mitochondria were abundant. Cell organelles all displayed intact structures (Figure 11.5) (Su et al., 2012).

The morphology of the digestive tract in aestivating *A. japonicus* was closely related to the stage of aestivation. Four stages were accurately defined: active period, initial aestivation period, deep aestivation period, and arousal period (Su et al., 2012). The investigators conducted a quantitative analysis of

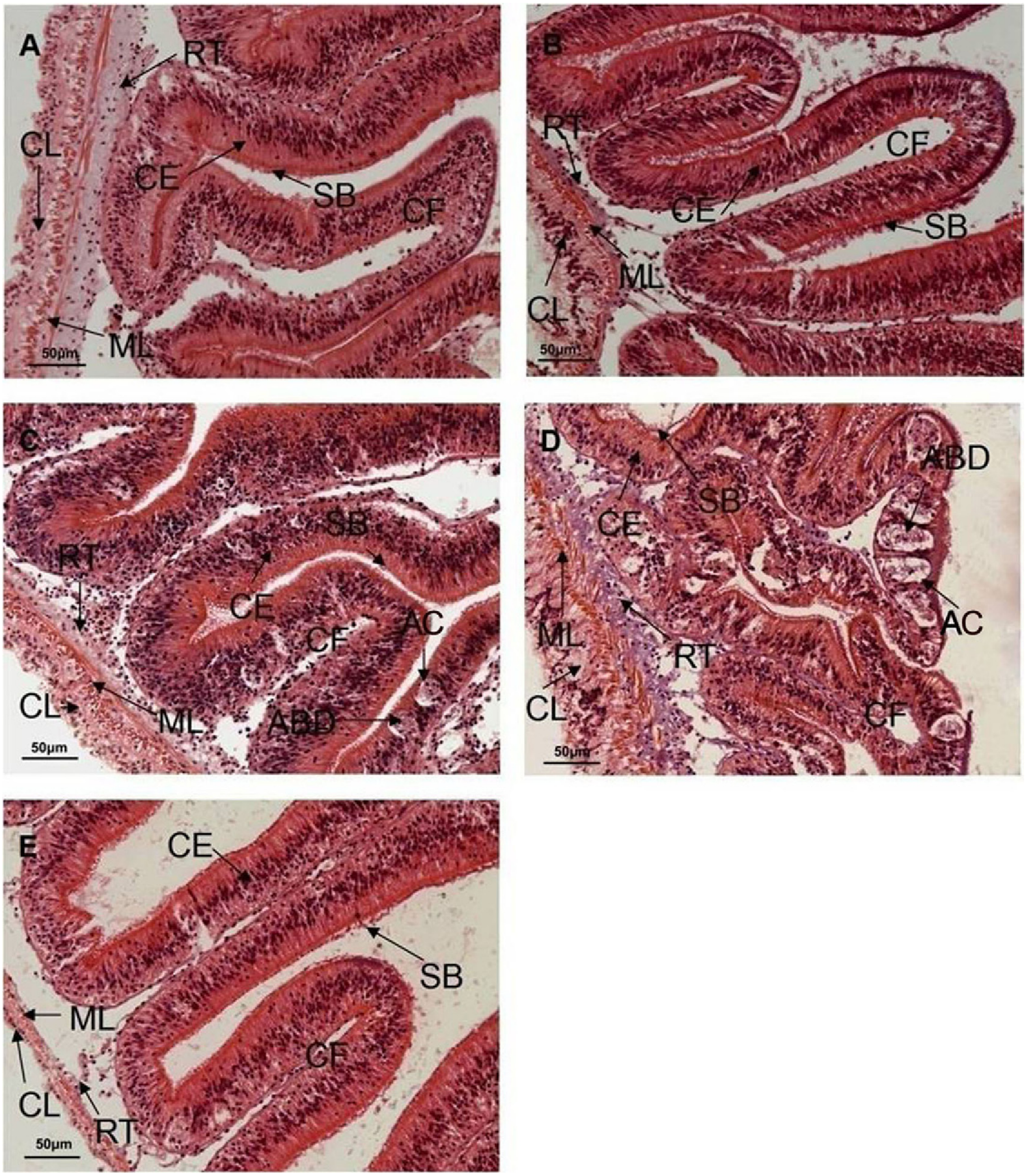

FIGURE 11.3

Light micrographs (A–E) with H&E staining of *A. japonicus* anterior intestines. (A) Anterior intestine sampled from control group at the onset. (B, C, D, and E) Anterior intestines sampled in experimental animals cultured at 25 °C for 0 d (A), 5 d (B), 10 d (C), and 20 d (D). CE, columnar epithelium; SB, striated border; CF, circular fold; RT, reticular tissue; ML, muscular layer; CL, coelomic lining; AC, apoptotic cavities; ABD, apoptotic body-like debris.

From Wang, 2011

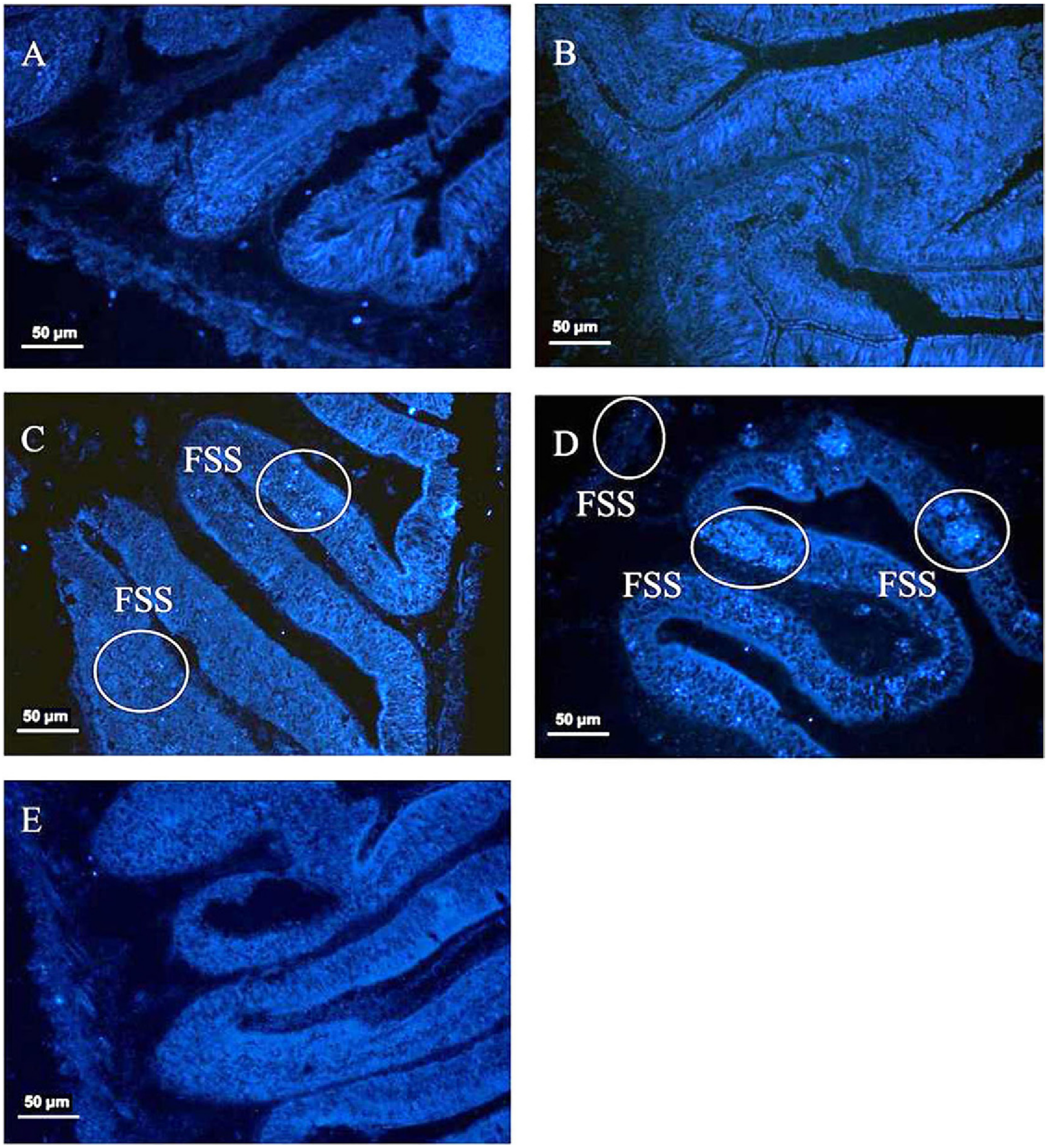

FIGURE 11.4

Fluorescent micrographs of TUNEL *in situ* assay of *A. japonicus* anterior intestines. (A) Anterior intestine sampled from control group at the onset. (B, C, D, and E) Anterior intestines in experimental animals cultured at 25 °C for 0 d (A), 5 d (B), 10 d (C), and 20 d (D). Micrographs in B and C reveal the fluorescent signal spots (FSS) of TUNEL positive cells.

From Wang, 2011

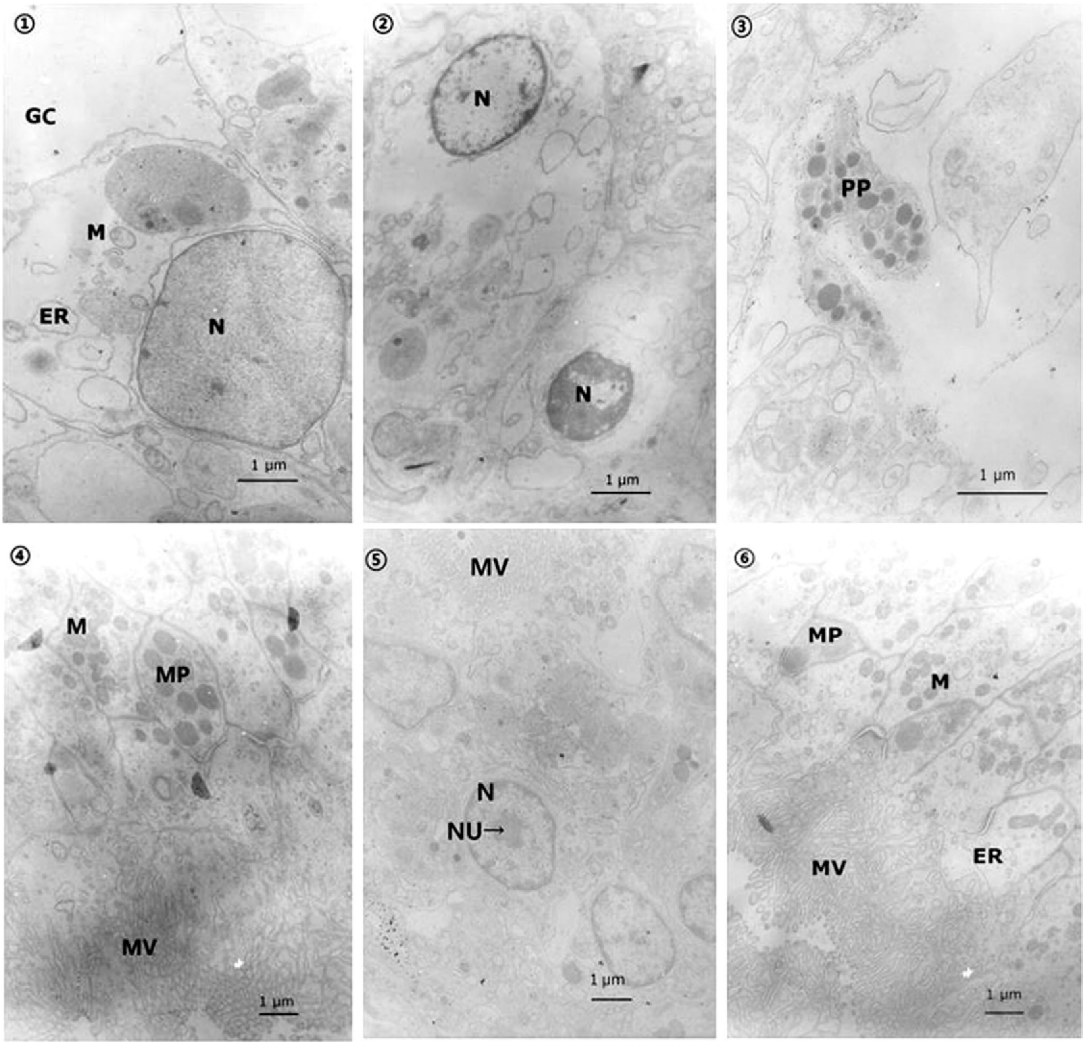

FIGURE 11.5

Transmission electron micrographs of anterior intestine mucous epithelium in *A. japonicus* during aestivation (①, ②, ③) and in active individuals (④, ⑤, ⑥). GC, gastrovascular cavity; M, mitochondria; ER, endoplasmic reticulum; N, nucleus; PP, pigmented particle; MP, mucous particle; MV, microvilli; NU, nucleoli.

From Su, 2012

the thickness of the anterior intestine tissue layer during aestivation. Their results showed that thickness of the submucosa did not vary among the different aestivation periods compared with controls, while mucosal epithelium and adventitia were thinner in the initial period (Su et al., 2012). Epithelial and muscle layers were thinner in the deep aestivation period. Finally, the anterior intestine diameter was smaller during the deep aestivation period (Table 11.1). Calculating the proportion of each layer of the

Table 11.1 Tissue Layers Thickness (μm) of Anterior Intestine at Different Stages of Aestivation in *A. japonicus*

	Mucosal Epithelium	Submucosa	Muscle Layer	Adventitia
Control	$46.29^{a} \pm 6.03$	$31.67^{a} \pm 9.89$	$10.08^{a} \pm 1.51$	$19.21^{ab} \pm 3.34$
Initial stage of aestivation	$23.21^{b} \pm 1.13$	$43.27^{a} \pm 2.54$	$10.32^{a} \pm 1.83$	$7.76^{c} \pm 2.65$
Deep aestivation	$25.95^{b} \pm 10.05$	$31.24^{a} \pm 15.97$	$4.66^{b} \pm 3.16$	$16.61^{bc} \pm 7.52$
Arousal stage of aestivation	$43.36^{a} \pm 4.62$	$49.10^{a} \pm 32.24$	$11.49^{a} \pm 2.20$	$23.13^{a} \pm 7.72$

Note: Letters indicate significant differences among treatments (mean ± SE; n = *5,* P < *0.05).*
From Su, 2012

intestinal wall revealed that the thickness of mucosal epithelium and adventitia accounted for less in the initial aestivation period than in the nonaestivation period, whereas the proportion of the submucosa was higher. In addition, the proportion of the muscle layer in the deep aestivation period was lower than in other periods (Table 11.2) (Su et al., 2012).

Apart from morphological changes in the digestive tract, the activity of digestive enzymes varies markedly during aestivation with an associated impact on intestinal functions. Gao et al. (2009) induced *A. japonicus* into aestivation and assessed the activities of amylase, lipase, trypsin, and other enzymes in the digestive tract. A decrease in the activity of amylase, lipase, and trypsin were found, while the activity of pepsin increased (Gao et al., 2009).

In summary, based on morphological analysis and enzymatic activity, it is now understood that the digestive tract of *A. japonicus* undergoes important changes during aestivation. Studies indicate that the rapid degradation of the sea cucumber's digestive tract leads to functional depression of the digestive system during aestivation, and to consequent reduction in energy consumption and storage.

Table 11.2 Proportional Thickness (%) of the Different Layers of the Anterior Intestine Wall at Different Stages of Aestivation in *A. japonicus*

	Mucosal Epithelium/ Wall Thickness	Mucosa/Wall Thickness	Muscle Layer/ Wall Thickness	Adventitia/Wall Thickness
Control	$43.32^{a} \pm 5.66$	$29.08^{b} \pm 6.80$	$9.48^{a} \pm 1.68$	$22.53^{a} \pm 7.76$
Initial stage of aestivation	$27.53^{b} \pm 1.84$	$51.26^{a} \pm 1.45$	$12.15^{a} \pm 1.20$	$12.87^{b} \pm 5.41$
Deep aestivation	$34.40^{ab} \pm 12.51$	$38.91^{ab} \pm 15.64$	$5.76^{b} \pm 3.43$	$22.74^{a} \pm 11.95$
Arousal stage of aestivation	$35.91^{ab} \pm 7.71$	$36.39^{b} \pm 9.35$	$9.44^{a} \pm 2.11$	$19.77^{a} \pm 4.91$

Note: Letters indicate significant differences among treatments (mean ± SE; n = *5,* P < *0.05).*
From Su, 2012

11.1.4 HYPOMETABOLISM AND REGULATION OF GENE EXPRESSION

The metabolic activity (based on oxygen consumption and ammonia excretion rates) of *A. japonicus* is much lower than normal during aestivation. Moreover, sea cucumbers with different body weight react differently according to the various ambient temperatures. The metabolic rate of large (148.5 ± 15.4 g) and medium-sized (69.3 ± 6.9 g) sea cucumbers peaked at 20 °C and then decreased as ambient temperature increased; the metabolic rate of small (21.2 ± 4.7 g) sea cucumbers peaked at 25 °C; however, the oxygen–nitrogen ratio of different-sized sea cucumbers did not vary during the different stages of aestivation (Yang et al., 2006). Yuan et al. (2007) suggested that the energy budget of different sizes of sea cucumber was adjusted in the aestivation period; oxygen consumption by large and small sea cucumbers during that period (30 °C) were respectively reduced by 54.4 and 79.7% compared with active sea cucumbers (15 °C). In the active period, the largest component of energy expenditure was defecation, accounting for more than 50% of the energy. The next most important energy expenditure was respiratory energy consumption, accounting for 19.8–39.4%. The lowest proportions of energy were consumed for growth and excretion, respectively accounting for 5.7–10.7% and 2.9–3.7%. During aestivation, the proportion of energy taken up by respiration and excretion were higher (to 88.3 and 13.6% of feed energy, respectively), while the proportion of total feeding energy expended on growth reached a negative value (−55.3%). Thus, *A. japonicus* exhibited a negative growth trend. Moreover, energy expended for feeding and defecation dropped to zero during aestivation. Therefore, previously stored energy in the body had to be mobilized in order to maintain basic physiological processes, such as respiration and excretion (Yuan et al., 2007).

Research on gene expression regulation during aestivation is of great importance to determine the mechanism underlying hypometabolism. Wang et al. (2011) used 454 high-throughput sequencing and preliminarily analyzed gene expression characteristics in *A. japonicus* during aestivation. Results showed that the expressed sequence tags (EST) library of active *A. japonicus* contained 165,848 of the original sequences, and the average sequence length of EST was 381.3 base pairs (bp); while the EST library of aestivated *A. japonicus* contained 137,396 of the original sequences and the average length of EST was 383.4 bp. After assembling, annotating, and gene ontology (GO) sorting, 2667, 2549, and 2668 sequences from the active group database were defined as unigenes and respectively allocated to molecular function, biological processes, and cellular components. In turn, 1866, 1807, and 1933 sequences from the aestivation database were respectively assigned to the same three GO categories. Then, sequences belonging to biological processes, cell components, and molecular function were respectively analyzed by secondary GO clustering. Corresponding gene sequences (Reads) of *A. japonicus* were statistically analyzed in aestivation and nonaestivation databases, and genes belonging to all categories were compared in both libraries. The aestivation library exhibited less gene copies and smaller proportions ascribed to molecular binding and catalytic activity, compared with gene expression in the database of active *A. japonicus*. Furthermore, there were less gene copies and smaller proportions involving biological regulation in the aestivation library. Moreover, the number of gene copies and proportions related to extracellular synthesis were also reduced in the aestivation library (Figure 11.6). Overall, 241 gene expressions were downregulated during aestivation, which was greater than the upregulated genes (93 sequences). A further analysis of 20 upregulated and downregulated genes indicated that gene coding for enzymes and proteins involved in catalysis, binding, and physiological regulation were mostly downregulated. The housekeeping genes (ribosomal protein) were moderately upregulated (Tables 11.3 and 11.4). Based on the analysis of comprehensive GO gene

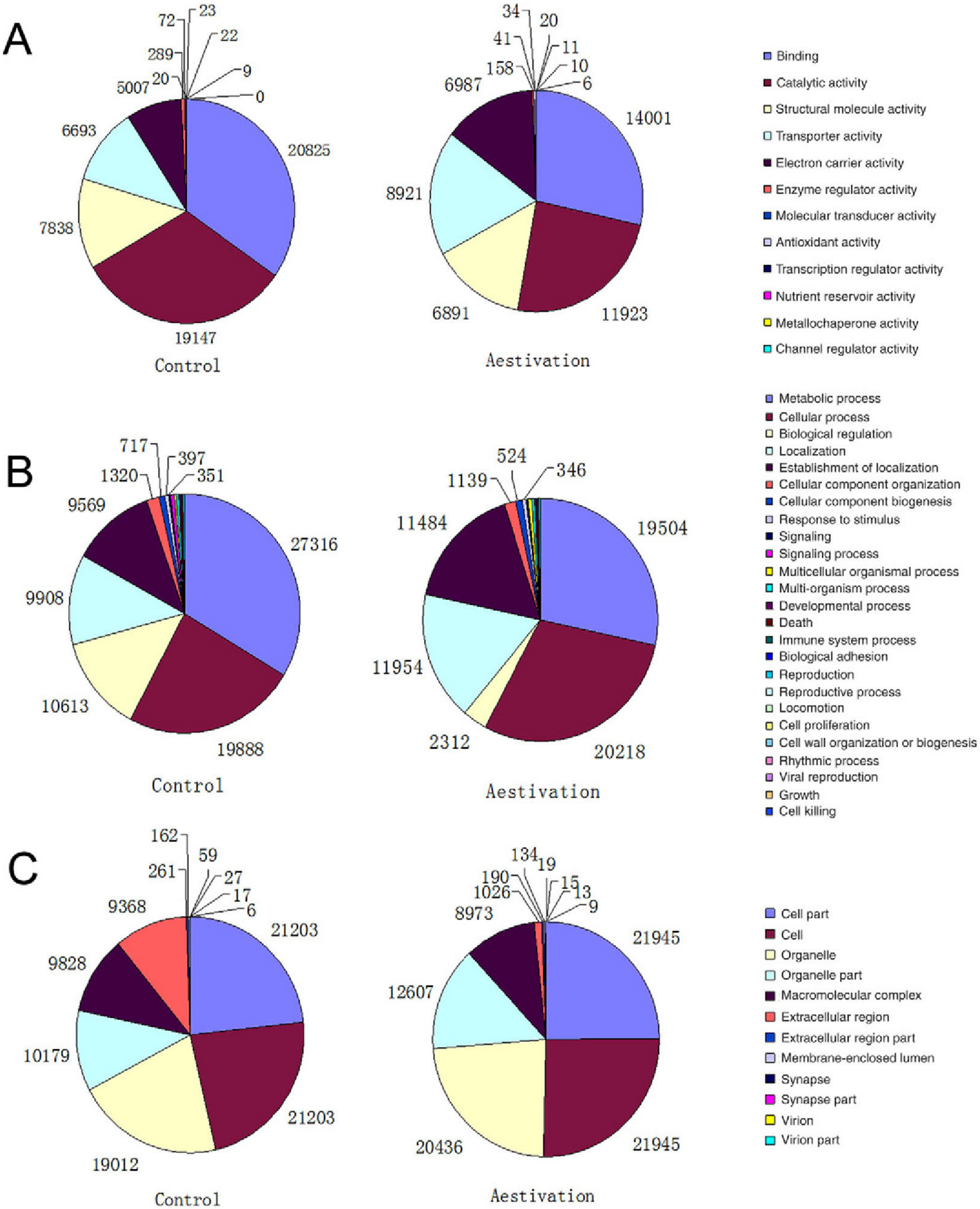

FIGURE 11.6

Distributions of the gene ontology terms (second level) across three categories in *A. japonicus*: A: molecular function, B: biological process, C: cellular component. Because one sequence can be attributed to more than one term, the sum of all terms is larger than the number of reads in each group of GO annotated unigenes.

From Wang, 2011

Table 11.3 Top 20 Downregulated Genes During Aestivation in *A. japonicus*

Annotated Gene Name	Genbank ID	Depths in Control	Depths in Aestivation	Fisher_*P*
Aqualysin-1	JI981124	7815	377	0
60S ribosomal protein L10	JI981125	1329	116	2.37E-151
Pancreatic triacylglycerol lipase	JI981126	303	3	6.67E-72
Carboxypeptidase A	JI981127	428	24	8.65E-63
Placental protein 11	JI981128	219	4	7.35E-51
Alkaline serine exoprotease A	JI981129	146	5	1.95E-32
40S ribosomal protein S3a-A	JI981130	89	0	1.02E-24
60S ribosomal protein L38	JI981131	295	84	1.03E-20
Dynein light chain Tctex-type 1	JI981132	80	1	1.07E-20
Soma ferritin	JI981133	1258	684	6.06E-15
Alpha-amylase 1	JI981134	50	0	3.66E-14
Cathepsin L1	JI981135	71	7	6.76E-13
Epidermal retinol dehydrogenase 2	JI981136	33	0	1.14E-09
WAP four-disulfide core domain protein 3	JI981137	62	9	1.40E-09
Fatty acid-binding protein, liver	JI981138	26	0	6.66E-08
Leucine-rich repeat-containing protein	JI981139	23	0	4.52E-07
60S ribosomal protein L22	JI981140	41	6	9.00E-07
Ras-related protein Rab-11B	JI981141	59	14	1.05E-06
Arginine kinase	JI981142	123	49	1.42E-06
Myophilin	JI981143	21	0	1.63E-06

From Wang, 2011

cluster and gene expression abundance, it was proposed that sea cucumber genes, most of which were nonhousekeeping genes involved in various activities, were suppressed during aestivation. The results were consistent with another study suggesting that sea cucumber metabolism is inhibited and life activity is reduced during aestivation (Wang et al., 2011).

Epigenetics is an important pathway of regulation of gene expression. It is also an important regulatory mechanism of various life processes, such as development, differentiation, growth, and disease occurrence (Fraga et al., 2007). Currently, epigenetic regulation is considered to be associated with hypometabolism in dormant animals by suppressing gene expression in hypometabolic systems.

Epigenetic regulation during *A. japonicus* aestivation has been analyzed by Wang et al. (2011) who screened eight genes related to epigenetic factors. Through induced aestivation and field surveys, gene expression variation involved in DNA methylation, chromatin remodeling, histone acetylation and histone methylation in intestinal tissue of aestivated *A. japonicus* were detected and analyzed. Results showed that expression of DNA (cytosine-5)-methyltransferase 1 (*DNMT1*), Methyl-CpG-binding domain

Table 11.4 Top 20 Upregulated Genes During Aestivation in *A. japonicus*

Annotated Gene Name	Genbank ID	Depths in Control	Depths in Aestivation	Fisher_*P*
Ubiquitin	JI981144	221	484	9.02E-23
Cytochrome *c* oxidase subunit 1	JI981145	4192	6089	1.55E-22
NADH-ubiquinone oxidoreductase chain 5	JI981146	159	379	7.99E-22
60S ribosomal protein L3	JI981147	104	248	7.92E-16
60S ribosomal protein L18	JI981148	52	159	5.08E-15
60S ribosomal protein L26	JI981149	302	514	5.12E-14
40S ribosomal protein S20	JI981150	29	105	1.64E-12
Cytochrome *b-c*1 complex subunit 7	JI981151	13	64	3.07E-10
60S ribosomal protein L19	JI981152	152	265	2.03E-09
40S ribosomal protein S15a	JI981153	150	252	2.27E-08
60S ribosomal protein L7	JI981154	65	135	5.00E-08
40S ribosomal protein S19	JI981155	44	105	5.81E-08
40S ribosomal protein S2	JI981156	220	333	9.57E-08
ATP synthase subunit a	JI981157	805	1017	2.01E-07
60S ribosomal protein L8	JI981158	82	153	2.85E-07
40S ribosomal protein S9	JI981159	60	122	3.89E-07
60S ribosomal protein L11	JI981160	169	261	5.68E-07
40S ribosomal protein SA	JI981161	16	55	6.82E-07
40S ribosomal protein S18	JI981162	223	312	7.59E-06
60S acidic ribosomal protein P1	JI981163	94	154	1.52E-05

From Wang, 2011

protein 2 (*MBD2*) and Chromodomain-helicase-DNA-binding protein 5 (*CHD5*) genes in relation to DNA methylation were upregulated to different degrees in aestivated sea cucumbers. Expression of Histone deacetylase 3 (*HDAC3*) and Histone-binding protein RBBP4 (*RBBP4*) genes related to histone acetylation were also upregulated to varying degrees. The expression of the Histone acetyltransferase KAT2B (*KAT2B*) gene remained unchanged. Expressional levels of HDAC3 rose in the intestines, which were sampled on the fifth and tenth day after the water temperature increase, and thereafter declined, but remained higher than in the control group. The expression of RBBP4 in the intestine showed an increase in samplings made on the twentieth and fortieth days of induced aestivation. The Histone-arginine methyltransferase CARMER (CARMER1) and Histone-lysine *N*-methyltransferase MLL5 (MLL5) genes related to histone methylation were clearly upregulated in intestinal tissue of aestivated sea cucumbers as well. Expression of related genes followed similar trends (Wang et al., 2011).

To explore the mechanism of DNA methylation in aestivated sea cucumbers, Zhao et al. (2013) cloned the key genes *DNMT1* and *MBD2/3* and analyzed their expression profiles. Results showed that expression

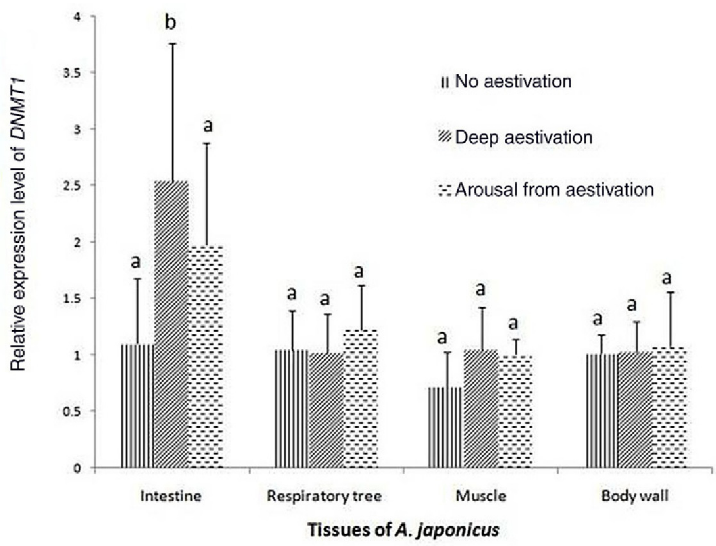

FIGURE 11.7

Relative expression levels of *DNMT1* in various tissues during aestivation in *A. japonicus.* Values were normalized against *NDUFA13*. Different lowercase letters indicate significant differences (b, $P<0.05$; c, $P<0.01$; e, $P<0.001$). Values indicate the mean ± SE ($N=6$).

From Zhao et al., 2013

of *DNMT1* was upregulated (2.5×) in intestinal tissue of deep aestivated sea cucumbers. The expression of *DNMT1* was unchanged in other tissues and other periods of the aestivation (Figure 11.7). Thus, it could be speculated that the *DNMT*1 gene, which is involved in DNA methylation, was regulated and that DNA methylation was affected in the intestine of *A. japonicus.* The expression of *MBD2/3* was upregulated in deep aestivated *A. japonicus* and reached 2.6× of the activity measured in sea cucumbers outside the aestivation period. Activity of the *MBD2/3* gene was also higher in tissues of the respiratory tree during deep aestivation and arousal period; as for other tissues, no changes were detected (Figure 11.8) (Zhao et al., 2013).

In summary, the metabolic rate of aestivated *A. japonicus* was decreased markedly. A generalized silencing of gene expression occurred. Only a few of the housekeeping genes and functional genes maintained a high level of expression or exhibited a slight increase. Epigenetic regulation of gene expression was apparent, possibly associated with the global suppression of gene expression during aestivation.

11.1.5 PROSPECTS

Since the first study on sea cucumber aestivation was published by Mitsukuri (1897), our knowledge of this process has developed considerably. In recent years, more research using ecological, morphological, physiological, molecular, and genomic methods were carried out to gain a deeper understanding of the regulatory mechanisms of aestivation. Currently, the value of these studies mainly lies in developing optimized breeding of high-temperature strains of sea cucumbers and, ultimately, in the establishment of efficient culture strategies throughout the year. Moreover, the theoretical value of findings on aestivation will presumably extend as *A. japonicus* emerges as a model organism for research on aestivation in the Echinodermata.

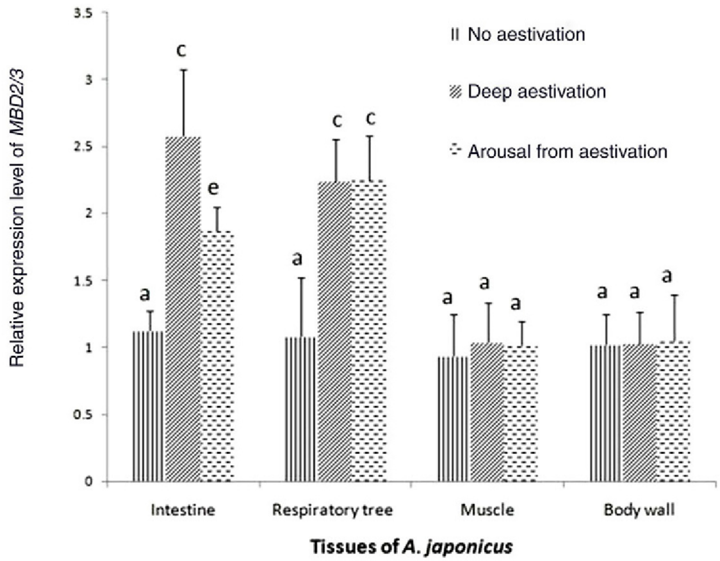

FIGURE 11.8

Relative expression levels of *MBD2/3* in various tissues during aestivation in *A. japonicus*. Values were normalized against *NDUFA13*. Different lowercase letters indicate significant differences (b, $P<0.05$; c, $P<0.01$; e, $P<0.001$). Values indicate the mean ± SE ($N=6$).

From Zhao et al., 2013

We believe that the following aspects of sea cucumber aestivation research should be investigated in the future:

1. The transcriptional profile of aestivating *A. japonicus* should be analyzed in-depth using transcriptomic research tools. Gene expressional regulation mechanism could be expanded from gene expression comparison, functional analysis of noncoding region and analysis of transcript structure.
2. More research on epigenetic regulation should be carried out to explore the mechanism of gene expression regulation in sea cucumber aestivation. The activities of epigenetic factors and levels of epigenetic modifications could be examined. Combined with the results of transcriptional regulation research, the process of epigenetic modification in aestivating *A. japonicus* could be expounded, helping us to get a deeper understanding of the hypometabolism of aestivation.
3. Hypometabolism in *A. japonicus* aestivation has been confirmed, but studies on metabolic regulation mechanisms would enrich the hypometabolism regulation theory in echinoderms and provide reference data to explore the evolution of metabolic regulation. Reversible phosphorylation and signal transduction regulation of carbohydrate, fat, and protein metabolism and ion channel activity are considered to play important roles in material and energy metabolism. These aspects should be studied to explore the corresponding regulation in sea cucumber aestivation.
4. Cell signaling regulates numerous physiological processes, and may play important roles in physiological adjustments during aestivation, such as tissue degeneration, metabolic rate depression, and immune system modification. Research on specific cell signaling in *A. japonicus* is expected to help resolve the mechanisms underlying aestivation.

11.2 REGENERATION

Regeneration is widely but nonuniformly represented among metazoans. Regenerative capacity varies among animal species, and generally increases from higher to lower life forms (Alvarado and Tsonis, 2006; Brockes et al., 2001). All animals are capable, to some extent, of tissue repair, which allows them to overcome environmental stresses, physical trauma, or diseases. Some species have limited repair capacity that may be restricted to the healing of wounds. Other species, such as echinoderms, possess a striking capacity to regenerate most of their lost tissue or organs. In particular, sea cucumbers are capable of regenerating the intestine, respiratory tree, gonads, and body wall (Dolmatov et al., 1996; Shukalyuk and Dolmatov, 2001; Dolmatov and Ginanova, 2009). They are thus considered as excellent models for organ regeneration studies. Some sea cucumbers that are exposed to stressful circumstances will eviscerate a more or less significant portion of their internal organs (Figure 11.9). The pattern of evisceration varies among species of sea cucumbers (García-Arrarás and Greenberg, 2001). Most, including those in the order Aspidochirotida, such as *Holothuria glaberrima* (Holothuridae) and *A. japonicus* (Stichopodidae), eviscerate their intestine, hemal system, and respiratory tree through the cloaca (Mashanov and García-Arrarás, 2011). However, other species, such as the dendrochirote *Eupentacta fraudatrix*, lose their viscera (mainly intestine) through the mouth (Vladimir and Yu, 2001). In addition, the viscera of *H. difficilis* are evacuated by muscular action through a tear in the body wall (Kille, 1937). The time needed for viscera to regenerate also varies from species to species. For example, it takes only seven days for the regeneration process to complete in *H. scabra* (Bai, 1971); while *Thyone briareus* (Kille, 1935), *A. japonicus* (Zheng et al., 2006b), and *H. glaberrima* (García-Arrarás et al., 1998; Mashanov and García-Arrarás, 2011) need ~30 days. *Stichopus mollis* requires even longer, up to 145 days (Dawbin, 1948). So far, studies on regeneration in sea cucumbers have mainly centered on *H. glaberrima*, *Eupentacta fraudatrix*, *A. japonicus*, *and H. forskali.* The regeneration of *A. japonicus* has mainly been studied over the past 10 years.

11.2.1 MACRO-ANALYSIS OF INTESTINE REGENERATION

The digestive tract is composed of a short and slim esophagus, a stomach, a light brown anterior intestine with many folds (accounting for one-quarter of the intestine), a straight middle intestine

FIGURE 11.9

Evisceration of the sea cucumber *A. japonicus* within 2 min after injection with 0.35 M KCl in the coelom. The sea cucumber eviscerated the intestine, hemal system, respiratory tree, and gonads from the cloaca.

From Sun, 2013

(accounting for one-quarter of the intestine), a tortuous posterior intestine (accounting for half of the intestine), and the cloaca, suspended to the body wall by muscle fibers (Figure 11.10A). The intestine is attached to the body wall by the dorsal, lateral, and ventral mesenteries (Figure 11.10A) (Wang and Li, 2007). During evisceration, rupture of the digestive tract occurs at the level of the stomach and the junction between the posterior intestine and the cloaca (Figure 11.10A).

In *A. japonicus*, the process of digestive tract regeneration in terms of morphology can be divided into the following five stages: wound healing, formation of blastema, lumen formation, intestine differentiation, and intestine growth.

1. Wound healing (0–2 days post-evisceration, dpe) (Figure 11.10B): After eviscerating, the edge of the mesentery is torn and appears thin, transparent, and irregular. The free edge is gradually repaired within 2 dpe and becomes complete.
2. Formation of blastema (2–5 dpe; Figure 11.10C): The dorsal and lateral mesenteries gradually thicken into a solid rod-like structure. The lateral and dorsal mesenteries heal to about one-third of the anterior part of the whole body, whereas the ventral and lateral mesenteries are connected by a transverse membrane. The closed gastric stump thickens and proliferates posteriorly along the dorsal mesentery. The remnants of the cloaca develop toward the front of the body to form a thin intestine rudiment, light yellow in color.
3. Lumen formation (5–14 dpe; Figure 11.10D): Lumen formation begins from the stomach and the cloaca, respectively, which are located at the two opposite ends of the intestine. At about 14 dpe, the two ends of the lumen fuse into a slim and fragile intestinal canal; at this point, some undigested food particles can be observed.
4. Intestine differentiation (14–21 dpe; Figure 11.10E): Tissue layers of the intestine gradually develop into a complete structure with digestive function. The intestine has differentiated into three parts (anterior, middle, and posterior) and has a typical "S" structure.
5. Intestine growth (21 dpe; Figure 11.10F): During this stage, the intestine continues to enlarge until it is gradually restored to its original size. The rate of intestine regeneration may be affected by many factors such as feeding conditions and individual variance.

11.2.2 MICROSCOPIC AND CELLULAR EVENTS DURING INTESTINE REGENERATION

The intestinal wall of the sea cucumber consists of four layers: luminal epithelium (mucosa), inner connective tissue (submucosa), muscular layer, and coelomic epithelium (serosa) (Figure 11.11A). Refer to Chapter 4 for anatomical details.

11.2.2.1 Formation of the intestinal lumen

As the mesentery thickens, a solid intestine develops and gradually enlarges into a regular tube-like structure (Sun, 2013). At 3 dpe, there is no obvious lumen in the intestine of *A. japonicus* (weight: 80–100 g); only some small cavities are visible (Figures 11.11B and 11.15C). At 7 dpe, the small cavities gradually develop into a large central space (Figures 11.11C and 11.15E). The connective tissue inside the intestinal lumen disappears gradually, and the lumen takes shape at ~10 dpe (Figures 11.11D and 11.15G). The intestinal lumen gradually expands and provides space for the luminal epithelium to develop (Figures 11.11E and 11.15I). The lumen appears at ~4 dpe and its development is very fast; in less than a week the intestine connects from two tubular outgrowths. The digestive tract develops a continuous lumen and the sea cucumbers begin to feed at ~16 dpe (Figure 11.12).

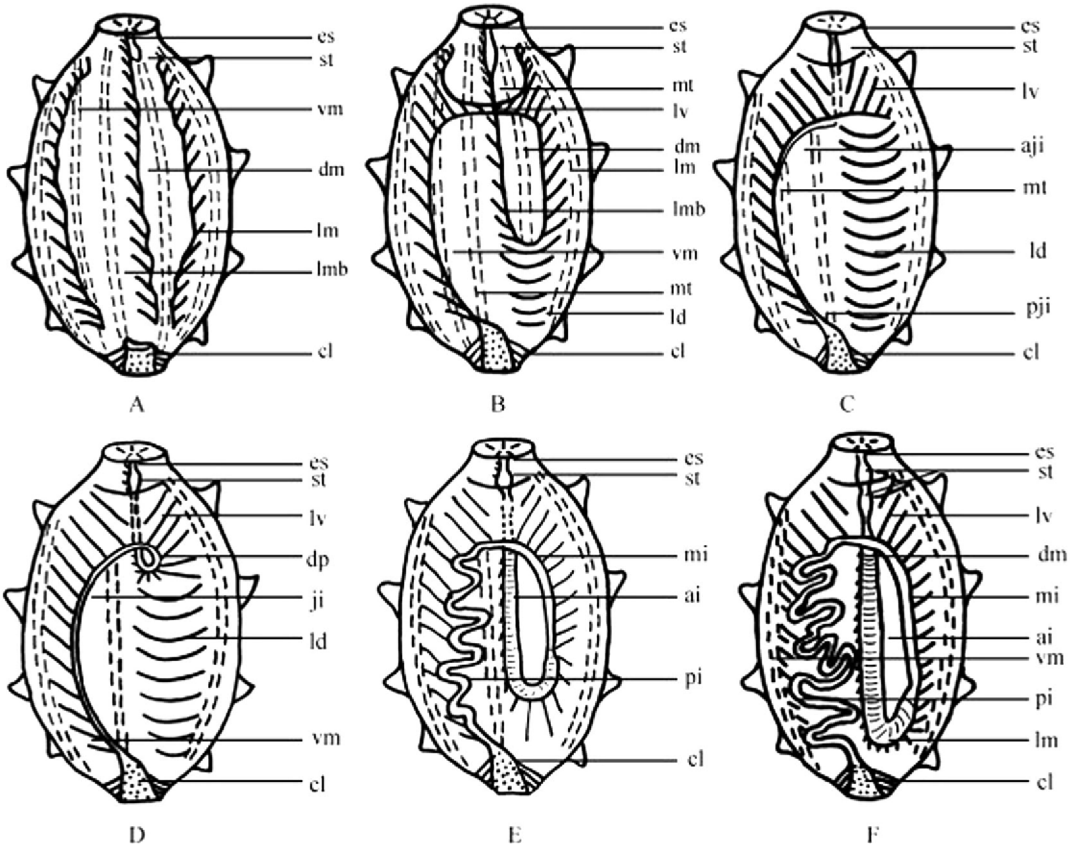

FIGURE 11.10

Regeneration of the digestive tract of the sea cucumber *A. japonicus.* (A) The normal digestive tract, which consists of six parts: esophagus, stomach, anterior intestine, middle intestine, posterior intestine, and cloaca. (B) Morphology of the digestive tract after evisceration. The torn edges of the mesentery in the coelom were free after evisceration. However, the wound on the mesenteric edge healed quickly. (C) Formation of the blastema. The mesenteric edge gradually thickened to form the blastema. (D) Formation of the intestinal lumen. The newly formed intestinal lumen originated from two ends (the esophagus and cloaca; indicated by arrows). (E) Differentiation of the intestine. The regenerated intestine developed into tissue with complete digestive function. (F) The growing intestine. The regenerated intestine grew to the size of the normal intestine prior to evisceration. es, esophagus; st, stomach; ai, anterior intestine; mi, middle intestine; pi, posterior intestine; vm, ventral mesentery; dm, dorsal mesentery; lm, lateral mesentery; cl, cloaca; aji, anterior regenerating intestine; dp, down-loop; ji, regenerating intestine; ld, the junction between lateral and dorsal mesentery; lmb, longitudinal muscle band; lv, the junction between lateral and ventral mesentery; mt, thickened mesentery edge; pji, posterior regenerating intestine.

From Wang and Li, 2007

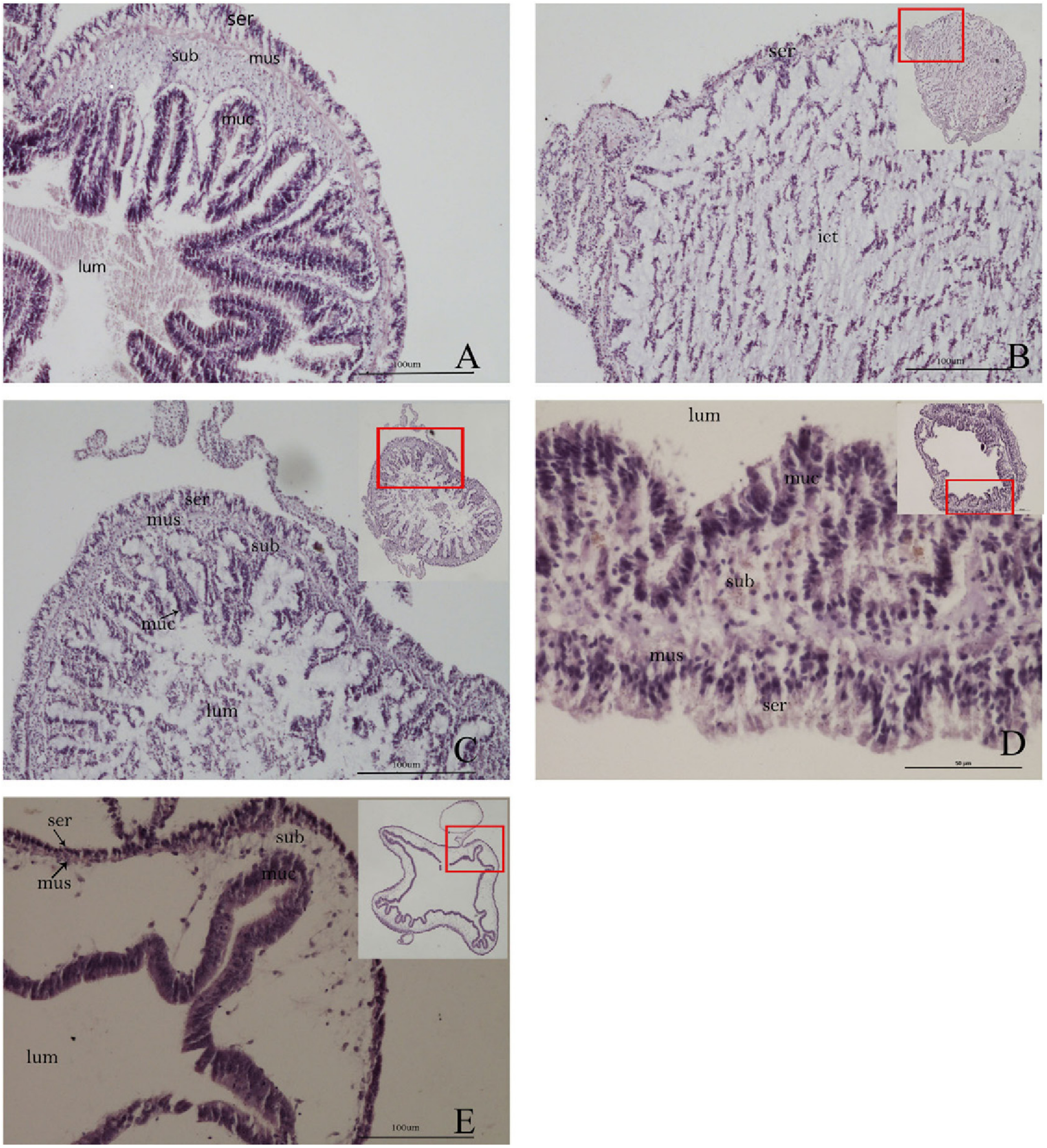

FIGURE 11.11

Histological sections of normal and regenerating intestine during the different regeneration stages in the sea cucumber *A. japonicus*. (A) Cross-section of normal intestine. From outside to inside, the intestinal wall is composed of serosa, muscle layer, submucosa, and mucosa. (B–E) Variation of intestinal tissue layers during four stages of intestinal regeneration at (B) three days post-evisceration (dpe), (C) 7 dpe, (D) 10 dpe, and (E) 14–21 dpe. ser, serosa; mus, muscle; sub, submucosa; muc, mucosa; lum, intestinal lumen.

From Sun, 2013

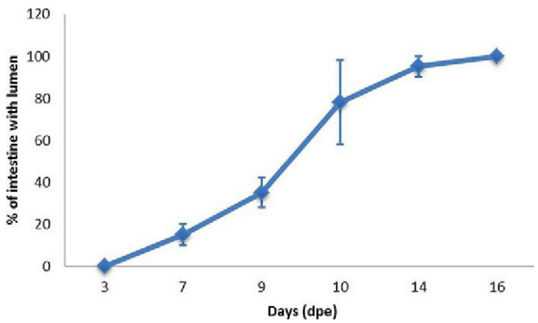

FIGURE 11.12

Time period required for lumen formation in *A. japonicus*. Data are reported as mean ± SE (N = 5).

From Sun, 2013

11.2.2.2 Formation of tissue layers of the intestine

The thickness of the four layers in the fully developed intestinal wall of *A. japonicus* is as follows: submucosa (accounting for 48%), serosa (accounting for 34%), mucosa (accounting for 11%), and muscular layer (7%) (Figure 11.13). In the early regenerating stage, the intestine wall is still undifferentiated. The muscle layer and mucosa form at ~7 dpe. At the beginning of regeneration, the coelomic epithelium (serosa) is derived from the mesentery and composed of a layer of cuboidal undifferentiated cells in a regular and close arrangement (Figure 11.14A). The undifferentiated cells differentiate into pinacocytes, whose proliferation and division thicken the coelomic epithelium (Figure 11.14B). Finally, the coelomic epithelium develops into a thicker epithelium consisting of loose connective tissue and a layer of cuboidal cells (Figure 11.14C).

A distinct muscular layer was observed in the newly formed intestine at 7 dpe (Figure 11.14C), and no obvious boundary between longitudinal and circular muscles was detected. At ~10 dpe, circular muscles were dominant in the muscular layer (Figure 11.14D). As intestine regeneration continued, circular and longitudinal muscles separated and appeared similar to those of fully developed and normal intestine.

The inner connective tissue was the main structure early on, accounting for nearly 96% of the newly developed intestine (Figure 11.13). It originated from the connective tissue of the mesentery. Formation of the luminal epithelial layer was strongly related to formation of the intestinal lumen. The intestinal lumen took shape by the microcavity fusing into a lumen. As the lumen formed, many undifferentiated cells assembled into the initial luminal epithelium (Figure 11.11B, C). Due to cell proliferation, the luminal epithelial layer gradually turned into a multilayered tissue. Meanwhile, undifferentiated cells developed into columnar cells with a striated border (Figure 11.14E). Finally, cells differentiated into long columnar cells, which formed the monolayer of columnar mucosa epithelium.

11.2.2.3 Cell division

The normal intestine of *A. japonicus* exhibits a stable state with few cell divisions (Figure 11.15A, B). At the early stage of intestine regeneration, dividing cells accounted for <3% in the serosa and inner connec-

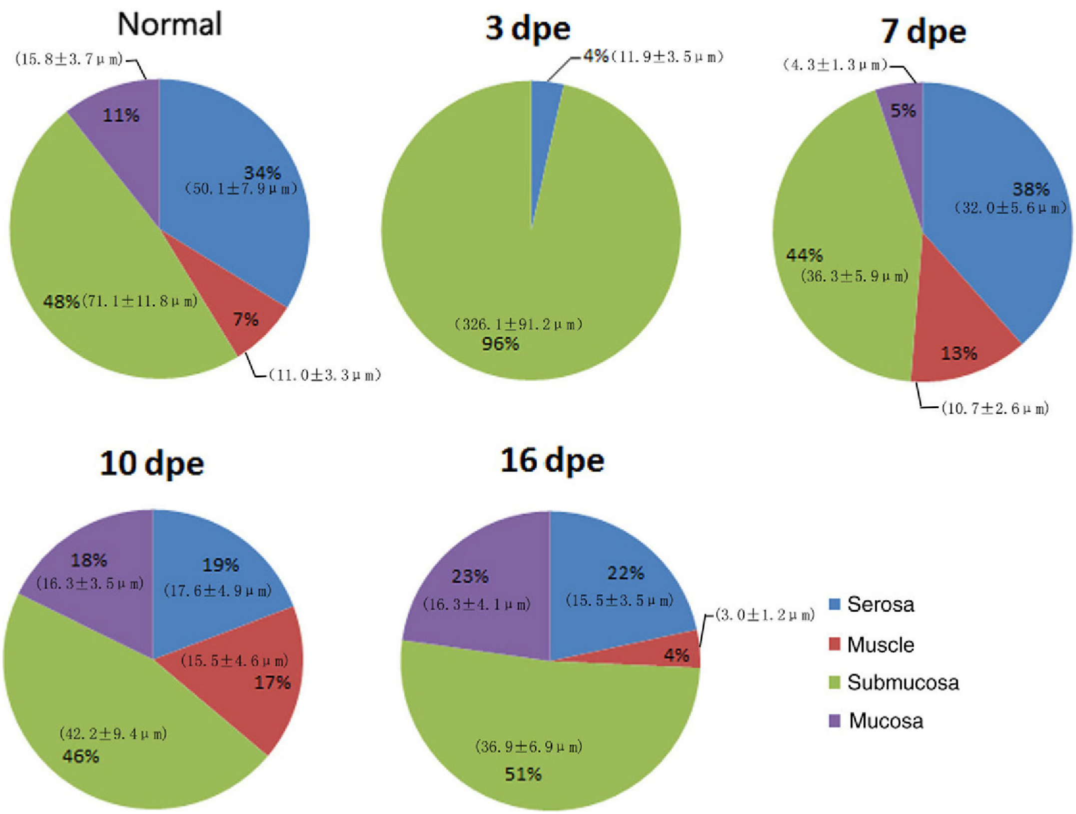

FIGURE 11.13

The distribution of the tissue layers in the normal and regenerating intestine at different stages in *A. japonicus*.

From Sun, 2013

tive tissue (Figure 11.15C, D). At 7 dpe, active cell division occurs in the luminal epithelium (29.5±3.1%) (Figures 11.15E, F and 11.16). At 10 dpe, cell division peaks in the luminal epithelium (37.9±3.11%). The percentage of dividing cells in the serosa and muscular layer increase to 19.9±2.7% (Figures 11.15G, H and 11.16). After 14–21 dpe, cell division decreases (Figures 11.15I, J and 11.16). Little cell division was observed during the early stage of intestine regeneration, but active cell proliferation activity appeared in the coelomic/muscular layer and the luminal epithelial layer at stages three (lumen formation) and four (intestine differentiation) of intestine regeneration (described earlier). Thus, intestine regeneration of *A. japonicus* mainly employs morphallaxis at the early stage and epimorphosis at a later stage.

11.2.2.4 Ultrastructure analysis

The fully developed intestinal epithelium of *A. japonicus* was composed of long columnar epithelial cells with developed microvilli and mucous cells (Figure 11.17A, B). Columnar epithelial cells are rich in mitochondria, which have strong digestion and absorption capacities. At the early stage of intestine regeneration, cell dedifferentiation was apparent in the esophagus and newly formed intestine: (1) material

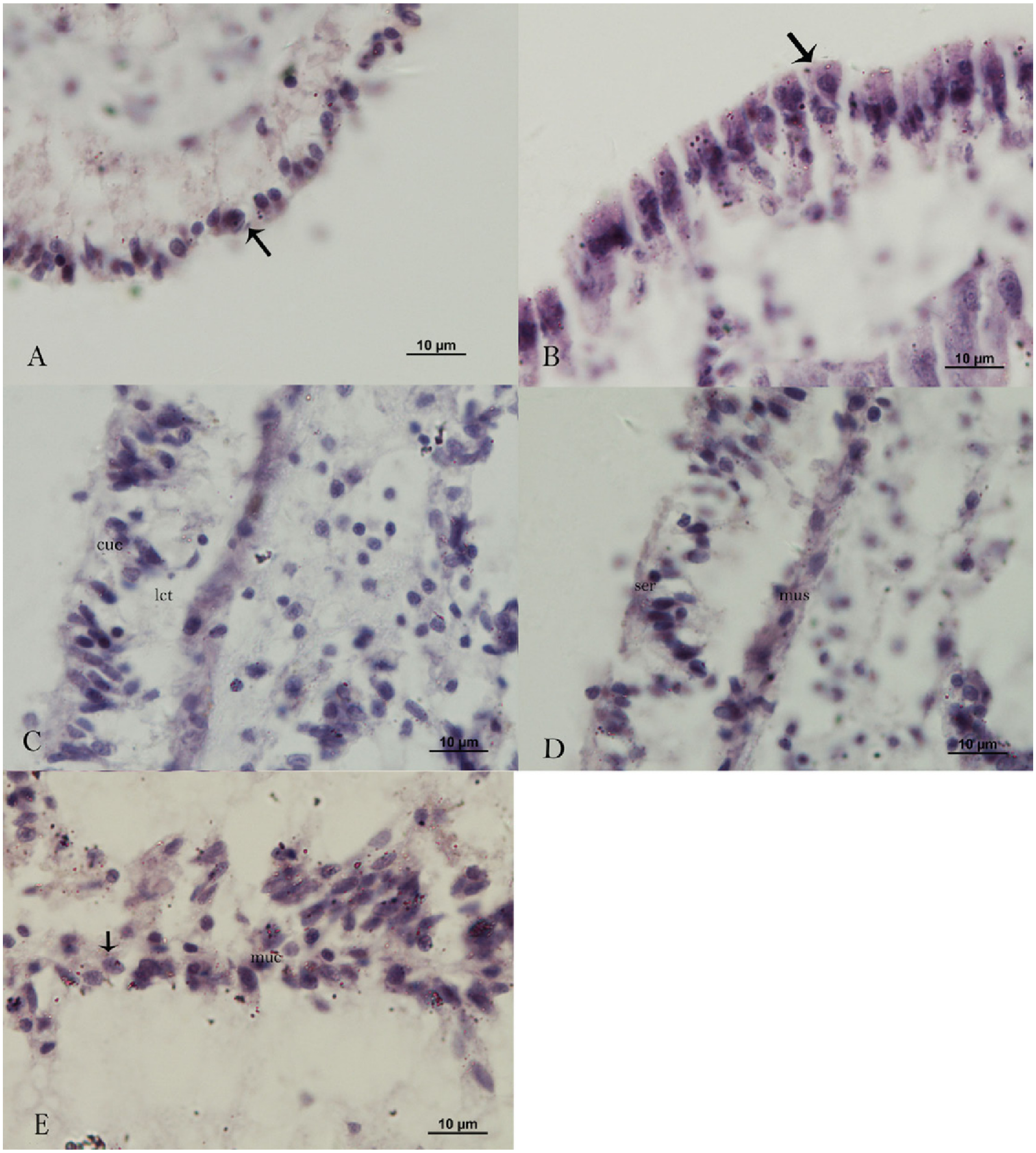

FIGURE 11.14

Cellular events in the process of tissue layer formation in *A. japonicus*. (A) Undifferentiated cells (arrow) in the serosa of the regenerative intestinal wall at three days post-evisceration (dpe). (B) Cuboidal cells (arrow) in the serosa of the regenerative intestinal wall at 7 dpe. (C) Two tissue layers (outer layer: a layer of cuboidal cells; inner layer: loose connective tissue) in the serosa of the regenerative intestinal wall at 10 dpe. (D) Newly formed muscle layer of the regenerative intestine at 7 dpe. (E) Epithelial cells in the mucosa of regenerated intestine at 7 dpe. cuc, cuboidal cells; ser, serosa; mus, muscle; muc, mucosa.

From Sun, 2013

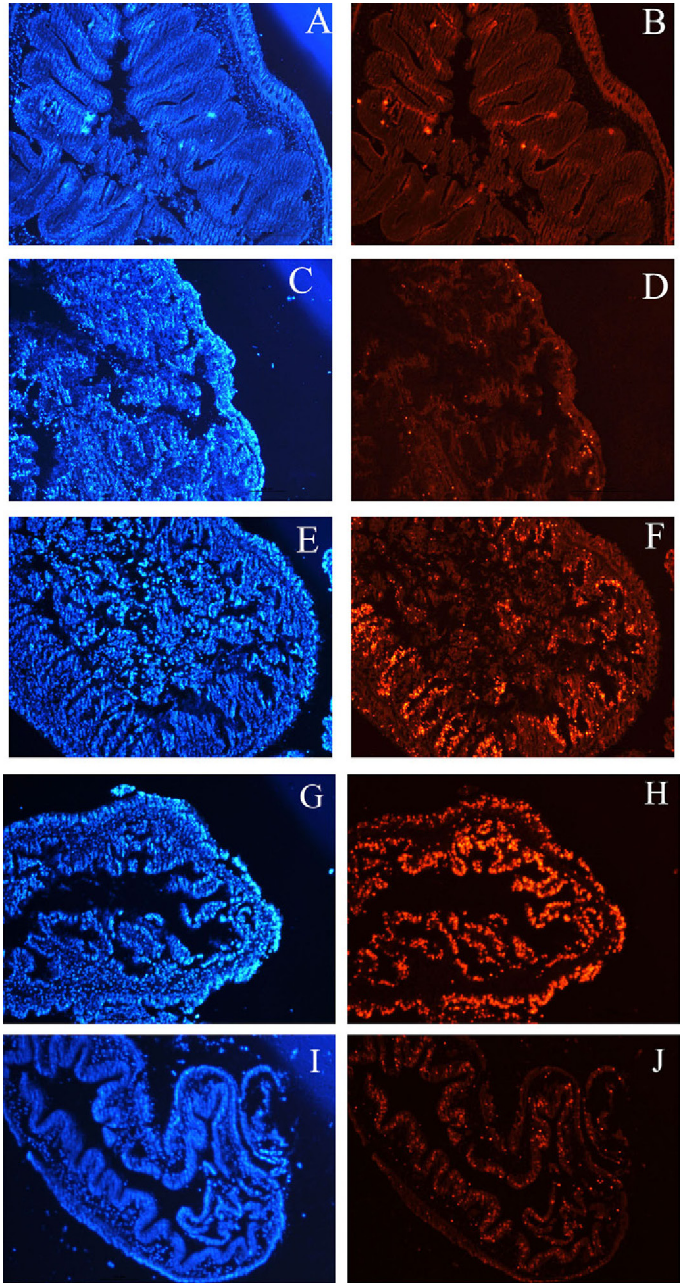

FIGURE 11.15

Double labeling with Hoechst (A, C, E, G, I) and EdU (B, D, F, H, J) in normal and regenerating intestine of *A. japonicus* at different stages of regeneration. A, C, E, G, and I are fluorescent images of cellular DNA (Hoechst stain, blue). B, D, F, H, and J are fluorescent images of replicated DNA (EdU labeling, red). The cells with red nuclei are descended from cells in the S phase and thus incorporated EdU into their DNA. (A and B) In cross-sections of normal intestine, dividing cell signals were rarely observed. (C and D) Less dividing cell signals were observed in the regenerative intestine at 3 dpe. (E and F) Most dividing cells were observed mainly in the luminal epithelium of regenerative intestine at 7 dpe. (G and H) Large numbers of dividing cells were observed in the luminal epithelium and serosa/muscle layer of regenerating intestine at 10 dpe. (I and J) The number of dividing cells gradually decreased, and they were located mainly in the luminal epithelium of regenerating intestine at 14–21 dpe.

From Sun, 2013

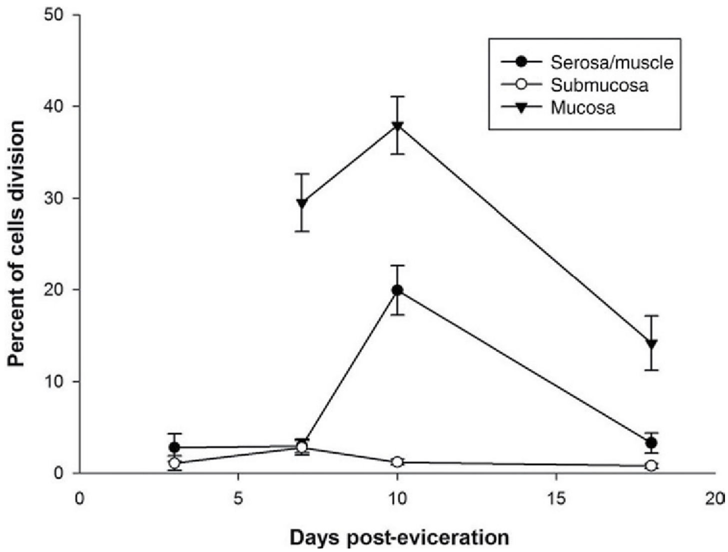

FIGURE 11.16

The pattern of cell proliferation in various tissue layers at different stages of regeneration in *A. japonicus*. Data are reported as mean ± SE ($N = 5$).

From Sun, 2013

within most cells was degraded or transferred, resulting in the formation of many vacuoles in the cells; (2) many vesicles formed by the cell inner membrane were observed in the cell plasma; and (3) the cell nucleus became large and was separated distinctly from the cell plasma (Figure 11.17C, D). In addition, fewer mitochondria were present in the epithelial cells of early regenerating intestine (Figure 11.17D).

The ruptured esophagus and cloaca healed into a blind tube and the free edge of the mesentery was neatly restored in preparation for the formation of new intestine. To date, most researchers agree that the thickening free edge of the mesentery plays a key role in intestine regeneration in sea cucumbers (Dawbin, 1948; Bai, 1971; García-Arrarás et al., 1998; Shukalyuk and Dolmatov, 2001; Candelaria et al., 2006; García-Arrarás and Greenberg, 2001). During the early stage of regeneration, changes in the mesentery involve major events: (1) mesenterial muscle dedifferentiation; (2) extracellular matrix remodeling; and (3) an increase in the number of spherule-containing cells (Quinones et al., 2002; Candelaria et al., 2006; San Miguel-Ruiz and García-Arrarás, 2007). During regeneration, cells in the connective tissues of the mesentery are the same type as those in the submucosa. In fact, the primordium of the regenerating gut originates from the thickening mesentery. Two theories about the origin of the primordium cells currently exist: (1) they are derived from dedifferentiation of the esophagus–stomach (Shukalyuk and Dolmatov, 2001; Mashanov et al., 2005) and (2) the dedifferentiating mesothelium serves as the initial source of rudiment cells (García-Arrarás et al., 2011; García-Arrarás and Greenberg, 2001). We detected obvious dedifferentiation in the esophagus and stomach of *A. japonicus*. Therefore, mesentery might be the path by which dedifferentiated cells in the esophagus migrate to newly formed tissue. However, some investigators have posited that the mesothelium that became a source of cells in repair and regeneration was a unique tissue layer (Dolmatov et al., 1994; García-Arrarás et al., 2011).

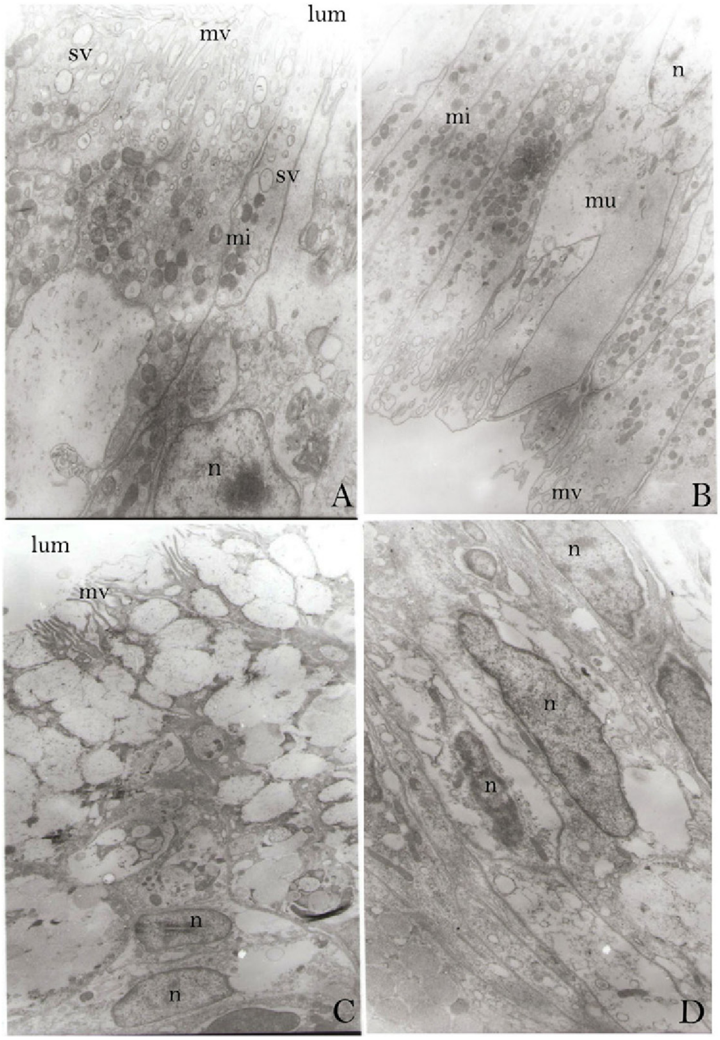

FIGURE 11.17

Transmission electron micrographs of the digestive tract of *A. japonicus*. (A and B) Ultrastructural organization of the normal digestive tract. (C and D) Ultrastructural organization of the digestive tract at three days post-evisceration (dpe). lum, lumen; mv, microvilli; sv, secretory vacuoles; mi, mitochondria; n, nucleus; mu, mucous cells. A: 6000×; B: 4000×; C: 4000×; D: 5000×.

From Sun, 2013

Mesenchymal stem cells are multipotent stem cells that can differentiate into a variety of cell types. García-Arrarás et al. (2011) reported that the cells in the mesothelium underwent a transition, especially at the injury site, and that mesothelial cells ingressed during intestinal rudiment formation (García-Arrarás et al., 2011). Based on these views, we propose that cells migrating from both the esophagus–stomach and the mesothelium contributed to mesentery thickenings (i.e. the blastema) in *A. japonicus*.

11.2.3 METABOLISM AND CHEMICAL COMPOSITION DURING VISCERA REGENERATION

11.2.3.1 Change in metabolic activity

From 0 to 10 dpe, body weight decreased in *A. japonicus*; at 10–15 dpe, body weight increased to some very little extent. At 15–25 dpe, body weight remained constant, no change was found; during 25–60 dpe, body weight increased gradually (Figure 11.18A) (Tan et al., 2008).

The average oxygen consumption of intact sea cucumbers was 25.96 ± 1.82 $\mu g\,g^{-1}h^{-1}$ during the experiment, whereas the oxygen consumption of eviscerated sea cucumbers decreased from 6.22 ± 1.20 $\mu g\,g^{-1}h^{-1}$ to the lowest value of 3.29 ± 1.39 $\mu g\,g^{-1}h^{-1}$ at 5 dpe, then increased slowly to normal levels (Figure 11.18B) (Tan et al., 2008).

The total ammonia excretion rate increased gradually during the regenerative process. At the start of the experiment the ammonia rate was 0.0060 ± 0.002 $\mu g\,g^{-1}h^{-1}$, which was merely 8.67% of the average ammonia excretion rate of the control (Figure 11.18C) (Tan et al., 2008).

During the viscera regeneration process, the ratio of oxygen to nitrogen (O:N) decreased to a relatively stable value with little fluctuation. At the start of the experiment, O:N was 90.5 ± 2.2, then it decreased until the end of the process, to as low as 34.1 ± 2.0. However, no significant difference was observed compared to control sea cucumbers whose O:N ratio was 33.2 ± 2.6 (Figure 11.18D) (Tan et al., 2008).

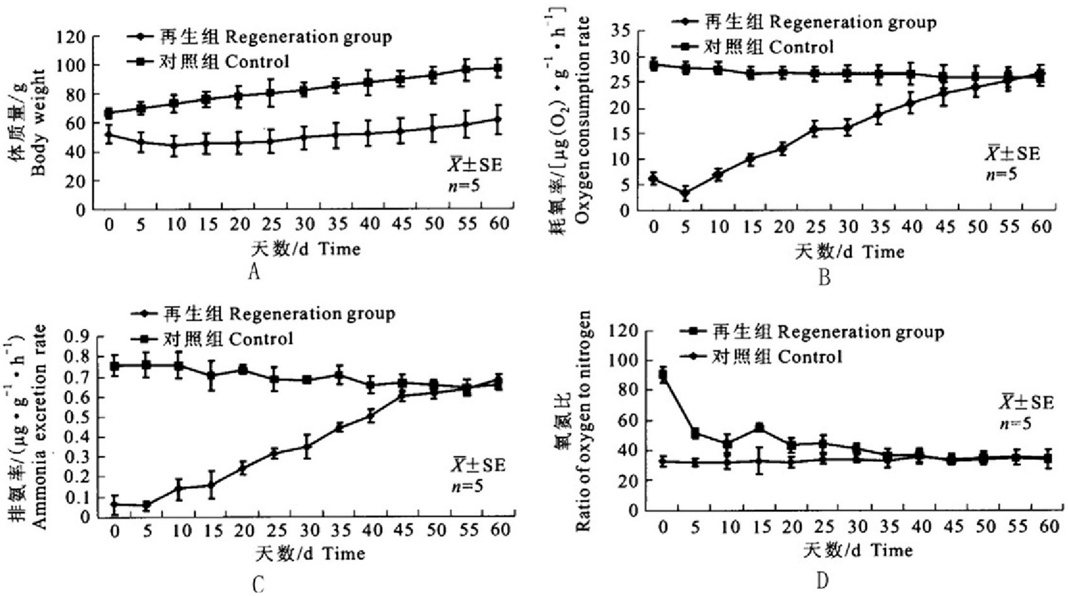

FIGURE 11.18

The body weight and biochemical change of *A. japonicus* during regeneration. (A) The body weight change of *A. japonicus* during regeneration. (B) Changes in oxygen consumption rate of *A. japonicus* during regeneration. (C) Changes in ammonia excretion rate of *A. japonicus* during regeneration. (D) Ratio of oxygen to nitrogen in *A. japonicus* during regeneration. Data are reported as mean ± SE (N = 3).

From Tan et al., 2008

11.2.3.2 Change in chemical composition

After full viscera regeneration, the crude protein, crude lipid, crude ash content, and energy ratio in *A. japonicus* were 2.35 ± 0.30, 0.93 ± 0.33, 3.65 ± 0.19, and 2.00 ± 0.12% (mean ± SE), respectively. These values were lower by about 16, 23, 5, and 25%, respectively, compared to the control group (Tan et al., 2008).

At the early stage of regeneration, in the absence of the respiratory tree, breathing is through the skin. With the development of viscera, especially after the restoration of functional digestion and respiration, metabolic activity rises, as shown by increasing oxygen consumption rates, and an overall increase in ammonia excretion rate, showing that in addition to the maintenance of physiological viability, energy provided for metabolism was needed for the regeneration of sea cucumbers (Tan et al., 2008).

During the regenerative process, O:N ratios were higher than 10, lipid and carbohydrate were consumed as energy materials, crude protein and crude lipid content also decreased, and ash content slightly increased. Lipid is an important component of animal cell membrane, cytoplasm, and nerve. During regeneration, lipids were consumed for high-intensity cell proliferation, resulting in a decrease in crude lipid in body chemical composition. Proteins play important roles in enzyme catalysis and hormonal regulation of physiological functions. Proteins may transform into lipids through deamination of amino acids, which can be used as energy reserve; therefore, proteins can transfer to lipids when the latter are consumed, leading to a decrease in protein content. The reason why ash content increased was because the relative ratio of ash to other chemicals was enhanced (Tan et al., 2008).

11.2.4 TRANSCRIPTOME DURING REGENERATION IN *A. japonicus*

Intestine regeneration is a process regulated by multiple genes, suggesting the inadequacy of investigating molecular mechanism through single gene analysis. Therefore, it is necessary to construct a cDNA library of intestine regeneration. Zheng et al. (2006a, b) constructed such a cDNA library including 327 EST (expressed sequence tag), which was the first work of this type on intestine regeneration in the sea cucumber *A. japonicus*. However, the traditional library fails to cover the whole gene transcription of intestine regeneration. High-throughput sequencing techniques like 454-life sequencing, solid, and solexa emerge as promising tools to construct a library.

Sun et al. (2011) obtained 165,848 and 182,473 raw reads by 454-life sequencing, which have been submitted to the NCBI (National Center for Biotechnology Information), under accession number SRA020994.2. For the control library, assembly of these trimmed, size-selected reads along with the publicly available EST sequences, 73% of the reads were assembled in 23,902 contigs of 381 bp in average length. The remaining 33,217 reads longer than 100 bp were retained as singletons, for a total of 57,119 unigenes. For the regeneration library, 70% of the reads were assembled in 24,867 contigs with an average length of 388 bp. The remaining 46,219 reads longer than 100 bp were retained as singletons, for a total of 71,086 unigenes. The average of sequencing coverage is 5.0853 and 5.133 for control and regeneration, respectively.

In the category "molecular function," the transcriptions of the genes associated with structural molecule activity were notably abundant in regeneration conditions compared with controls. This suggested that regeneration affects transcriptional responses. The dominant terms for "molecular function" are clearly binding, while the dominant terms for "biological process" are cellular and metabolic processes. Within "cellular component" the dominant terms are evenly divided between organelle, cell part, and cell (Sun et al., 2011).

Table 11.5 Genes from Essential Pathways Annotated in *A. japonicus* Unigene During Regeneration

Pathway ID	Pathways	Gene Name	Depth* in Control/ Depth in Regeneration	Fisher *p*
4010	MAPK signaling pathway	*TRAF* (TNF receptor-associated factor)	1/13	1.27E-07#
		ERK (extracellular signal-regulated kinase)	1/0	1.00E+00
4012	ErbB signaling pathway	*GRB2* (growth factor receptor-binding protein 2)	1/2	1.49E-01
4310	Wnt signaling pathway	*WNT6*	0/2	5.93E-02
		Frizzled	0/5	8.66E-04#
4330	Notch signaling pathway	Notch	13/15	1.23E-03#
		Delta	7/47	1.44E-21#
		TACE	2/3	9.69E-02
4340	Hedgehog signaling pathway	Slim b	1/1	4.27E-01
		Megalin	0/1	2.43E-01
4350	TGF-beta signaling pathway	*BMP* (bone morphogenetic protein)	0/12	4.74E-08#
		Noggin	0/3	1.45E-02#
4370	VEGF signaling pathway	*CDC42* (cell division control protein 42)	4/4	1.05E-01
		Paxillin	0/2	5.93E-02
4512	ECM-receptor interaction	Laminin	3/44	2.46E-23#
		Collagen	149/415	7.68E-103#
		Tenascin	164/213	7.05E-34#
4514	Cell adhesion molecules (CAMs)	*NCAM* (neural cell adhesion molecule)	5/3	4.12E-01
		SELP (selectin, platele)	0/1	2.43E-01
4630	Jak-STAT signaling pathway	*STAT* (signal transducer and activator of transcription)	0/2	5.93E-02
		PIM1 (proto – oncogene serine/threonine – protein kinase Pim-1)	2/3	9.69E-02

**Depth: the number of each gene reads in control library and regeneration library.*
#p < 0.05.
From Sun et al., 2013

Ten major intercellular signaling pathways were reported to play a key role in initiating and maintaining animal regeneration based on matches to the KEGG (Kyoto Encyclopedia of Genes and Genomes) database; 21 important genes involved in these signaling pathways were successfully identified in the transcriptome sequences. Some of these genes were significantly upregulated during regeneration compared to controls (Table 11.5) including Frizzled, Notch, Delta, BMP, Noggin, Laminin, Collagen, and Tenascin (Sun et al., 2011).

11.2.5 LARGE-SCALE EXPRESSION PROFILES OF INTESTINE REGENERATION

Intestine regeneration in *A. japonicus* involves five key stages (described earlier). This process can thus be described as a continuous dynamic change in gene expression that underlies the molecular mechanisms involved in regeneration. Sun et al. (2013) used RNA-Seq to determine the global dynamic changes in the gene expression profile during intestine regeneration in *A. japonicus*.

A total of 4,868,208, 4,727,453, 5,030,570, 4,715,682, and 4,877,984 reads were sequenced using RNA-Seq technique in Normal, 3, 7, 14, and 21 dpe libraries, respectively. After trimming the raw reads, 4,848,595, 4,708,854, 5,011,237, 4,696,545, and 4,855,872 clean reads were obtained in Normal, 3, 7, 14, and 21 dpe libraries, respectively (Sun et al., 2013).

A large number of genes were differentially expressed at a high level between normal and regenerative libraries, especially at the early stage of intestine regeneration. When compared to Normal, 2,415 upregulated genes (10.64%, 2,415/22,691) and 1,099 (4.84%, 1,099/22,691) downregulated genes were observed at 3 dpe, and 2,520 (11.11%, 2,520/22,691) upregulated genes and 1,084 (4.78%, 1,084/22,691) downregulated genes were screened at 7 dpe. The number and expression level of DEGs gradually decreased in the 21 dpe library, and only 1,012 (4.46%, 1,012/22,691) upregulated genes and 554 (2.44%, 554/22,691) downregulated genes were observed (Sun et al., 2013).

Key genes associated with the regenerative process were classified into three groups: developmental genes, extracellular matrix (ECM) associated genes, and cytoskeletal genes.

Regeneration and development share similar mechanisms, so developmental genes are excellent candidates for future study on the molecular mechanisms of regeneration (Jopling et al., 2011). Most genes (*Wnt*, *Hox*, *BMP*, and syndecan) were upregulated; while *krueppel-like6* was downregulated during regeneration in *A. japonicus* (Sun et al., 2013). In addition, genes from the same family showed different expression patterns. For example, *Hox1* and *Hox3* were upregulated at the early stage 3, 7, and 14 dpe and reached a peak at 3 dpe. No changes were observed for *Hox9/10* at 3 dpe, but its expression

Table 11.6 Expression Profile of Developmental Genes During Intestine Regeneration in *A. japonicus*

Gene ID	Gene Name	Normal (RPKM)	3 dpe (RPKM)	7 dpe (RPKM)	14 dpe (RPKM)	21 dpe (RPKM)
isotig19205	*Wnt4*	4.68	14.43↑	45.81↑	20.42↑	12.34↑
isotig18112	*Wnt6*	5.97	9.04↑	21.62↑	16.28↑	6.42
isotig14310	*Wnt8*	—	1.93	—	1.36	—
isotig24437	*Hox1*	8.77	33.80↑	26.82↑	18.59↑	12.38
isotig16174	*Hox3*	—	19.62↑	5.34↑	4.32↑	—
isotig23550	*Hox9/10*	2.75	3.97	21.60↑	22.47↑	21.98↑
isotig18972	*Hox 11/13*	—	—	0.80	1.67	3.46↑
isotig17483	*BMP*	21.38	22.88	48.41↑	48.91↑	39.49↑
isotig14688	*BMP1*	6.96	31.45↑	30.86↑	24.31↑	19.64↑
isotig03217	*syndecan*	170.41	271.40↑	214.40	172.68	219.38
isotig28523	*krueppel-like6*	31.99	19.81↓	21.95↓	20.76↓	47.30↑

↑: upregulated, ↓: downregulated RPKM (reads per kb million reads) represents relative expression level of gene.
From Sun et al., 2013

Table 11.7 Expression Profile of ECM (Extracelluar Matrix) Associated Genes During Intestine Regeneration in *A. japonicus*

Gene ID	Gene Name	Normal (RPKM)	3 dpe (RPKM)	7 dpe (RPKM)	14 dpe (RPKM)	21 dpe (RPKM)
isotig22120	*alpha-2 collagen*	13.41	63.41↑	86.25↑	110.75↑	95.21↑
isotig06513	*alpha-5 collagen*	43.46	148.89↑	236.37↑	265.37↑	299.74↑
isotig00683	*tenascin R*	—	2.26↑	4.09↑	5.68↑	0.73
isotig16822	*tenascin XB-like*	—	4.36↑	0.66	2.06↑	0.71
isotig15853	*laminin alpha*	12.97	107.46↑	96.88↑	58.33↑	25.63↑
isotig20868	*laminin gamma*	63.63	262.07↑	318.13↑	174.40↑	149.67↑
isotig07199	*MMP1*	0.35	1.36	3.08↑	1.60↑	0.33
isotig15379	*MMP12*	—	0.58	1.06↑	1.10↑	—
isotig19398	*MMP14*	6.67	0.92↓	2.50↓	6.06	5.38
isotig14227	*MMP16*	31.79	181.87↑	96.13↑	31.55	39.58
isotig13187	*MMP19*	4.18	12.37↑	51.13↑	47.75↑	57.60↑
isotig23432	*MMP20*	—	5.21↑	—	—	—
isotig22633	*spondin*	11.48	28.26↑	69.07↑	34.76↑	52.81↑
isotig09128	*fibropellin Ia*	0.57	1.09	2.47↑	1.54↑	1.07↑
isotig17742	*fibropellin Ib*	9.06	26.18↑	35.97↑	39.65↑	38.75↑
isotig19172	*papilin*	140.30	—	0.82↓	14.45↓	24.64↓

↑: *upregulated*, ↓: *downregulated RPKM (reads per kb million reads) represents relative expression level of gene.*
From Sun et al., 2013

remained at a high level from 7 to 21 dpe. In the case of *Hox11/13*, the upregulating trends occurred from 14 dpe (Table 11.6).

The ECM content undergoes significant changes during intestine regeneration and those changes are closely related to MMPs activity (Quinones et al., 2002). Six families of genes (*collagen, tenascin, laminin, MMPs, spondin*, and *fibropellin*) associated with ECM were screened in *A. japonicus* (Sun et al., 2013). All of these genes, except *MMP14*, upregulated at one or several stages of intestine regeneration in *A. japonicus* (Table 11.7).

All cytoskeletal genes showed changes at 3 or 7 dpe during intestine regeneration in *A. japonicus* (Table 11.8). Some (*alpha-tubulin*, *beta-tubulin*, and *actin*) were upregulated; while others (*gamma tubulin*, *myosin*, and *gelsolin*) were downregulated.

11.2.6 KEY GENES OF INTESTINE REGENERATION

The study on key genes of intestine regeneration in *A. japonicus* is still in its infancy, and it is only focused on the transcription of *WNT6*, *Hox6*, ependymin encoding (*epenAj*), and trypsin-like serine protease genes (Table 11.9) (Yuan et al., 2012; Zheng et al., 2006a). Therefore, more time must be given to the functional studies of these genes.

Table 11.8 Expression Profile of Cytoskeletal Genes During Intestine Regeneration in *A. japonicus*

Gene ID	Gene Name	Normal (RPKM)	3 dpe (RPKM)	7 dpe (RPKM)	14 dpe (RPKM)	21 dpe (RPKM)
isotig14228	*alpha-tubulin*	23.05	60.93↑	71.10↑	81.96↑	88.12↑
isotig22271	*beta-tubulin*	24.57	57.98↑	115.89↑	152.89↑	97.10↑
isotig23490	*gamma-tubulin*	23.12	9.17↓	23.76	18.53	16.64
isotig02880	*actin*	103.15	82.45	186.76↑	209.20↑	255.78↑
isotig07384	*actin 2*	73.99	144.74↑	230.59↑	244.80↑	321.89↑
isotig08506	*myosin III*	4.06	0.36↓	0.32↓	1.01↓	0.35↓
isotig15711	*myosin V*	33.06	22.05↓	23.88↓	20.21↓	38.28
isotig16090	*myosin VI*	63.12	12.94↓	20.53↓	27.45↓	21.48↓
isotig15703	*gelsolin*	1500.62	373.58↓	489.86↓	758.17↓	1014.86↓

↑: upregulated, ↓: downregulated RPKM (reads per kb million reads) represents relative expression level of gene.
From Sun et al., 2013

Table 11.9 Key Genes of Intestine Regeneration in *A. japonicus*

Genes	Full Length (bp)	Amino Acid (AA)
Wnt6	1140	342
Hox6	1312	321
Ependymin encoding gene (*epenAj*)	1119	263
Trypsin-like serine protease gene	1216	273

From Sun et al., 2013; Yuan et al., 2012; Zheng et al., 2006a, b

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CHAPTER

ALBINISM

12

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SUMMARY

Albinism is widespread in the animal kingdom and is caused by the absence of melanin in the hair, eyes, or skin. Research on albinism has mainly focused on humans and mice and there is little directed research on albino echinoderms. In this chapter, the occurrence and mechanisms of albinism in the sea cucumber *Apostichopus japonicus* are discussed, and we conclude as follows: (1) The lack of melanin in the body wall is the direct cause of albinism in *A. japonicus*; (2) the lack of melanocytes and melanin synthesis in melanosomes are histological characteristics of albino *A. japonicus*; and (3) the significantly low microphthalmia-associated transcription factor (*MITF)* and *astacin* expression levels in *A. japonicus* are the molecular causes of albinism.

Keywords: albinism; *Apostichopus japonicus*; *astacin*; melanin; melanocyte; microphthalmia-associated transcription factor; sea cucumber

12.1 RESEARCH ON ALBINISM

Albinism is widespread in the animal kingdom (Jiang, 2002), and is caused by the absence of melanin in the eyes, hair, and/or skin (Potterf et al., 1998). In the natural environment, wild albino individuals are very rare. Without protective coloration, they are conspicuous targets for predators. Furthermore, albino individuals normally suffer a series of pathological changes, including anemia, inner ear defects, megacolon, osteosclerosis, and nervous system disease (Zhao, 2011).

Research on albinism has mainly focused on humans and mice. In mammals, there are two types of pigment cells including eumelanin and phaeomelanin, both of which are derived from neural crest cells. The latter are a type of pluripotent stem cell, and the differentiation, proliferation, and distribution of pigment cells are strictly regulated. Albinism in mammals is an autosomal recessive disease, which results in the abnormal color of eyes, hair, and skin. In accordance with different characteristics, albinism in humans and mice is divided into two types: oculocutaneous albinism type 1 and type 2 (Beermann, 1990; Oetting, 2000; Prashiela et al., 2001).

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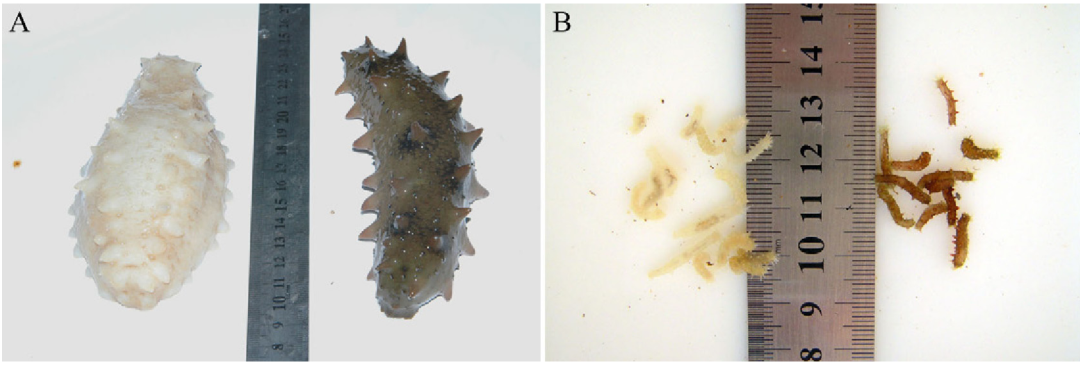

FIGURE 12.1

A. japonicus. (A) Albino adult (left) and normally pigmented individual (right); (B) juvenile albino individuals (left) and normal juveniles (right).

Photo by Heling Zhao

When compared to mammals, only few albino aquatic organisms have been studied, including Japanese rice fish (medaka), rainbow trout, zebra fish, goldfish, turbot, flounder, Chinese mitten crab, other crabs, and sea urchin (Arthur et al., 2005; Guo et al., 2007; Hyodo et al., 1997; Kajishima and Takeuchi, 1977; Kelsh and Schmid, 2000; Koga and Hori, 1997; Nakamura et al., 2001; Seikai, 1985). Like in mammals, pigments in fish are derived from neural crest cells. There are four types of pigments: melanophores, xanthophores, erythrophores, and iridophores. In accordance with their different distribution and abundance in skin, the color and pattern of aquatic animals vary.

To date, there has been limited directed research on albino echinoderms. As is typical in most echinoderms, coloration varies widely among individuals of the sea cucumber *Apostichopus japonicus*. In normal adult individuals, dorsal surfaces are dull yellowish-brown to maroon in color, and ventral surfaces are fawn to russet (Hyman, 1955). Very few individuals are albinos, having little or no pigmentation on their dorsal and ventral surfaces (Figure 12.1) (see also Chapter 3). From years of cultivating albino *A. japonicus*, we have come to the conclusion that they maintain normal growth rate, digestion rate, and fertility, when compared to normally colored individuals. Recently, sexually mature albinos used as broodstock produced albino offspring in captive breeding programs (Zhao, 2011; Lin et al., 2013), making *A. japonicus* an excellent biological model for the study of albinism.

12.2 BIOCHEMICAL AND GENETIC PATHWAYS UNDERLYING ALBINISM

The immediate cause of albinism is the absence of melanocytes or a disorder of melanin generation in the eyes, skin, and hair. Melanin generation is triggered by tyrosinase (TYR), and takes place in melanosomes. The main processes of melanin synthesis, secretion and metabolism include the following: (1) assembly of melanosome and synthesis of melanin in melanocytes, (2) transfer of melanosome from nucleus periphery to distal dendrites, (3) transfer of melanosome to keratinocyte, and (4) distribution and degradation of melanosome in keratinocyte. These processes are regulated by many factors, such as hormones, cytokines, and the physicochemical property of the environment surrounding melanocytes (Zhao, 2011; Wang, 2007; Wang et al., 2007; Lin and Fisher, 2007).

There are four main signal transduction pathways regulating melanin synthesis, secretion, and metabolism in melanocytes: adenylate cyclase/cAMP-dependent protein kinase pathway, mitogen activated protein kinase (MAPK) pathway, diacylglycerol/protein kinase C (DAG/PKC) pathway, and nitric oxide/cyclic guanosine monophosphate/protein kinase G (NO/cGMP/PKG) pathway. Among these pathways, the *TYR* gene family plays an important role in melanin synthesis, and the microphthalmia-associated transcription factor (*MITF*) is an essential regulator for melanocyte development, proliferation, and survival (Jiri and Jan, 2010; Schiaffino, 2010; Scott and Leopardi, 2003; Scott et al., 2007; Singh et al., 2005). Outside stimuli, such as α-melanocyte stimulating hormone (α-MSH), can regulate the transcriptional activity of *MITF* via a series of signaling pathways (e.g., cAMP and MAPK pathways). *MITF* can regulate the transcriptional activity of TYR genes and consequently regulate melanin generation (Zhao, 2011; Busca et al, 2000; Busca and Ballotti, 2000; Cheli et al., 2009; Park et al., 2004).

Tyrosinase, a metal enzyme containing copper, is a major enzyme that controls the speed of melanin synthesis. The *TYR* gene family encodes three functional enzymes involved in the melanin synthetic pathway, including TYR, tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2). The protein primary structures coded by these genes are very similar; they contain N-terminal signal sequence, C-terminal hydrophilic transmembrane domain, two Cys-rich domains and two His-rich domains (Zhao, 2011; Inagaki et al., 1994; Inagaki and Koga, 1998; Jimenez-Cervantes et al., 1994).

Research on the *MITF* gene has been limited primarily to vertebrates, particularly humans and mice. Mutations of *MITF* in albino humans or mice frequently exhibit a compromised region encoding the basic helix loop helix leucine zipper (bHLHZip) domain (Kazuhisa et al., 2000). Mutations in *MITF* are responsible for skin depigmentation, hearing damage, and abnormal iris pigmentation, which are caused by the absence or functional disorder of retinal pigment epitheliums (RPE) and melanocytes in the skin, inner ear, and eyes.

Furthermore, *MITF* is implicated as an important regulator of expression of the *TYR* gene family (Slominski et al., 2004; Takeda et al., 2000). The promoters of *TYR* genes share the M box with the core sequence CATGTG, which can be bound by *MITF*. Thus, *MITF* can *trans*-activate the expression of *TYR* genes, and consequently regulate melanin synthesis. Despite its importance, little is known about the role of *MITF* in albino *A. japonicus*.

12.3 DIFFERENCES IN NUTRIENT AND MELANIN CONTENTS BETWEEN ALBINO AND NORMAL *A. JAPONICUS*

In normal individuals of *A. japonicus*, juveniles develop pigmentation on their body wall when reaching about 1 cm in length. As they become adults, their dorsal surfaces typically turn dull yellowish-brown to maroon in color, and their ventral surfaces turn fawn to russet (Hyman, 1955), with different color variants known to occur (Chapter 3). In contrast, albino individuals have little or no pigmentation on their body wall from the early juvenile stage.

12.3.1 NUTRIENT CONTENTS

There is apparently no difference in the ash and moisture contents of the body wall between albino and normal adults of *A. japonicus*. The body wall of normal adults was determined to exhibit an ash content equivalent to 91.44 ± 0.27% (mean ± SD) of wet weight and a moisture content of 2.59 ± 0.07%, while in albinos, ash content was 90.29 ± 0.16% of wet weight and moisture content of 2.6 ± 0.03% (Zhao, 2011). Similarly, Sui (1998), Gao (2008) and Gao et al. (2009) reported that the moisture content in the

body wall of normal *A. japonicus* was about 90.8% on average. Among aquatic organisms, *A. japonicus* exhibits high ash content, as it has many small calcareous ossicles in its body wall. Gao (2008) reported that the ash content in the body wall of normal *A. japonicus* was 2.99–3.33% (3.22% on average), in accordance with the results of Zhao (2011) for both normal and albino individuals.

The total protein content in the body wall of adult albino *A. japonicus* was established at 51.1 ± 2.4% of dry weight, while in normal adults it was 49.6 ± 2.2% (Zhao, 2011) and 41.90–49.83% (Gao, 2008). The total protein content in the body wall of albino adults is therefore slightly higher than in normal adults.

The total lipid content in the body wall of adult albino *A. japonicus* was 4.2 ± 0.93% of dry weight, while the total lipid content in normal adults was 4.38 ± 1.25% (Zhao, 2011), similar to previous measures of 1.44–4.65% (Gao, 2008). The total lipid content in the body wall of albino adults is slightly lower than in normal adults. The abundance of food is the main factor affecting the total lipid content in the body wall of *A. japonicus*. The total sugar content in the body wall of adult albino *A. japonicus* was 5.51 ± 0.32% of dry weight, while it was 5.49 ± 0.33% in normal adults (Zhao, 2011) in accordance with values previously measured by Gao (2008; 3.08–5.34%).

12.3.2 MELANIN CONTENTS

Melanin generation is a complex biochemical reaction, and any factor affecting this complex reaction may cause albinism. In fish, the main morphological characteristic in partial or full albino skin is related to the dysfunctionality of melanin generation. Melanin content in the body wall of normal adults of *A. japonicus* was 3.12% dry weight, or 16 times greater than the melanin content in adult albino (0.24% dry weight) (Figure 12.2; Zhao, 2011). This supports the idea that the absence of melanin deposition in the body wall is the immediate cause of albinism in *A. japonicus*.

As the early juvenile stage is the critical period for pigmentation, normal and albino juveniles of *A. japonicus* were collected for melanin content analysis at 25, 32, 39, 46, 53, 60, 67, 74, 81, and 88 days postfertilization, and the melanin contents in their body wall determined and compared (Figure

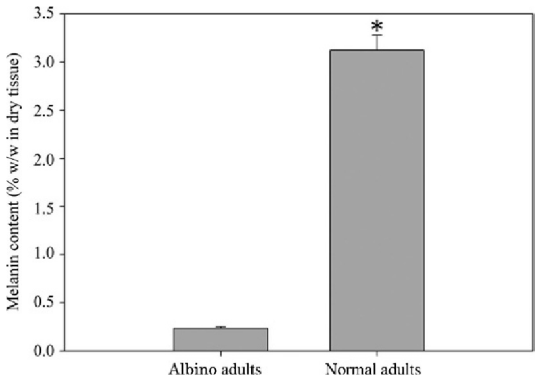

FIGURE 12.2

Melanin contents in the body wall of albino and normally pigmented adults of *A. japonicus*. The asterisk indicates significant differences ($P < 0.05$) between albino and normal individuals. Values in percent dry weight are shown as mean ± standard deviation ($n = 5$).

From Zhao, 2011

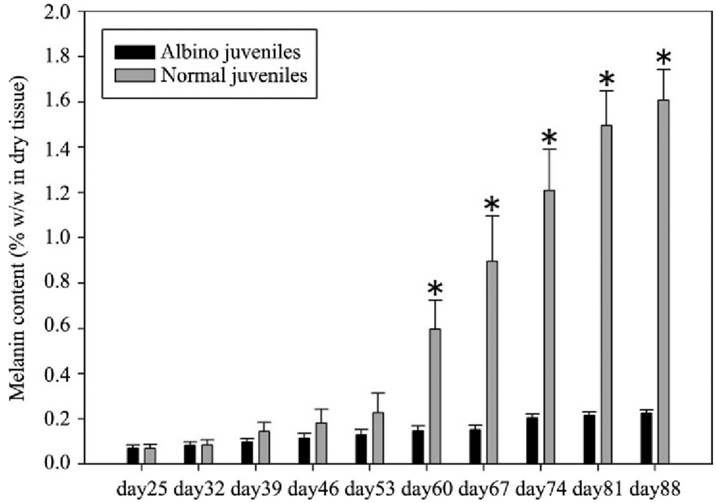

FIGURE 12.3

Melanin contents in juvenile albino and normally pigmented *A. japonicus* at various stages of development. The asterisk indicates significant differences ($P < 0.05$) between albino and normal individuals. Values in percent dry weight are shown as mean ± standard deviation ($n = 5$).

From Zhao, 2011

12.3, Zhao, 2011). Between 25 and 53 days postfertilization, there was no difference in melanin content in the body wall between albino and normal juveniles. By 60 days postfertilization, juveniles were about 7 mm in length and pigmentation appears in the body wall of normal juveniles, whereas the albino juveniles remain without pigmentation. At this time, melanin content in the body wall of normal juveniles (0.60 ± 0.13%) was already higher than in albino juveniles (0.15 ± 0.02%). Thereafter, melanin content in the body wall of normal juveniles continually and substantially increased, whereas melanin content in albino juveniles only slightly increased. By 88 days postfertilization, juveniles were about 1 cm in length and normal juveniles turned yellowish-green or reddish-brown, whereas there was little or no pigmentation in the body wall of albino juveniles. Melanin content in normal juveniles was 1.6 ± 0.14% and was much lower in albino juveniles at 0.23 ± 0.02%.

12.4 DIFFERENCES IN HISTOLOGICAL AND ULTRASTRUCTURAL CHARACTERISTICS BETWEEN ALBINO AND NORMAL *A. JAPONICUS*

12.4.1 HISTOLOGICAL AND ULTRASTRUCTURAL CHARACTERISTICS IN ADULTS

Thin sections from the body wall of adult *A. japonicus* that were stained with hematoxylin and eosin (H&E) discriminated the cuticle, epidermis, and dermis tissue, with no clear boundary between the epidermal and dermal layers, which is in accordance with Hyman (1955). The epidermal layer of albino adults was thinner relative to normal adults (Zhao et al., 2012a). Staining with Masson Fontana revealed that the melanocytes were primarily distributed in the epidermal layer. Albino adults had fewer

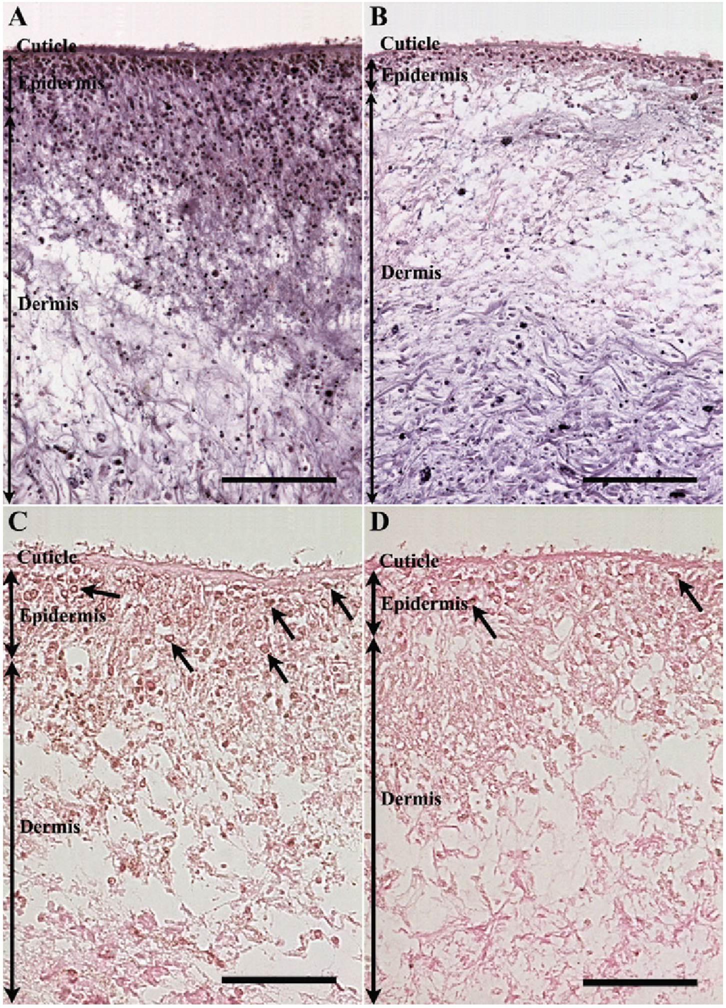

FIGURE 12.4

Histological cross-section of the body wall of normally pigmented (A and C) and albino (B and D) adult of *A. japonicus* with melanocytes (arrows) stained with H&E (A and B) and Masson Fontana (C and D). Scale bar (A and B) = 100 μm, (C and D) = 50 μm.

From Zhao, 2011

epidermal melanocytes compared with normal adults, which may be related to the thinner epidermal layer (Figure 12.4; Zhao et al., 2012a).

Electron microscope observations of normal adult epidermis revealed the ultrastructure of the mature melanocytes, which consisted primarily of developed melanosomes. Melanosomes were less dense in the melanocytes of albino adults compared with those of normal adults. Furthermore, the melanocytes of albino adults also contained nonpigmented vacuoles (Figure 12.5; Zhao et al., 2012a).

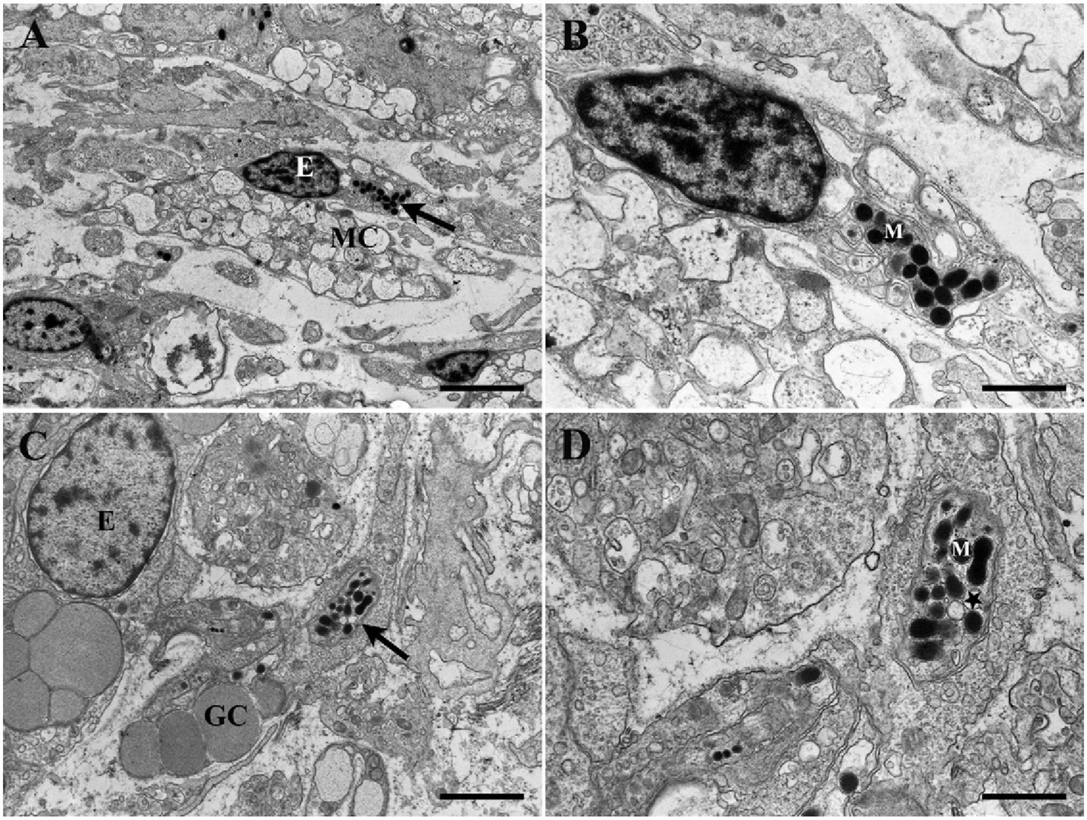

FIGURE 12.5

Electron micrographs of epidermal melanocyte (arrow) of normally pigmented (A and B) and albino (C and D) adult of *A. japonicus* (M, melanosomes; E, epithelial cell; MC, mucous cell; GC, granule-containing cell; ★ melanosome vacuoles). Scale bar (A and C) = 0.2 μm, (B and D) = 0.1 μm.

From Zhao, 2011

12.4.2 HISTOLOGICAL AND ULTRASTRUCTURAL CHARACTERISTICS IN JUVENILES

Like in adults, thin sections of the body wall of juvenile *A. japonicus* stained with H&E also discriminated the cuticle, epidermis, and dermis tissue. When compared with adults, the body wall of juveniles sampled 88-day postfertilization had a more loose structure (Zhao et al., 2012a). Normal juveniles had already accumulated a number of melanocytes in the basal layer of the epidermis, exhibiting incipient melanogenesis of the epidermal layer. However, albino juveniles had very few epidermal melanocytes during the same developmental phase (Figure 12.6; Zhao et al., 2012a).

The melanocytes of normal juveniles contained both developing and developed melanosomes, whereas the melanocytes of albino juveniles did not contain any developed melanosomes. Furthermore, the melanocytes of albino juveniles contained many nonpigmented vacuoles (Figure 12.7; Zhao et al., 2012a).

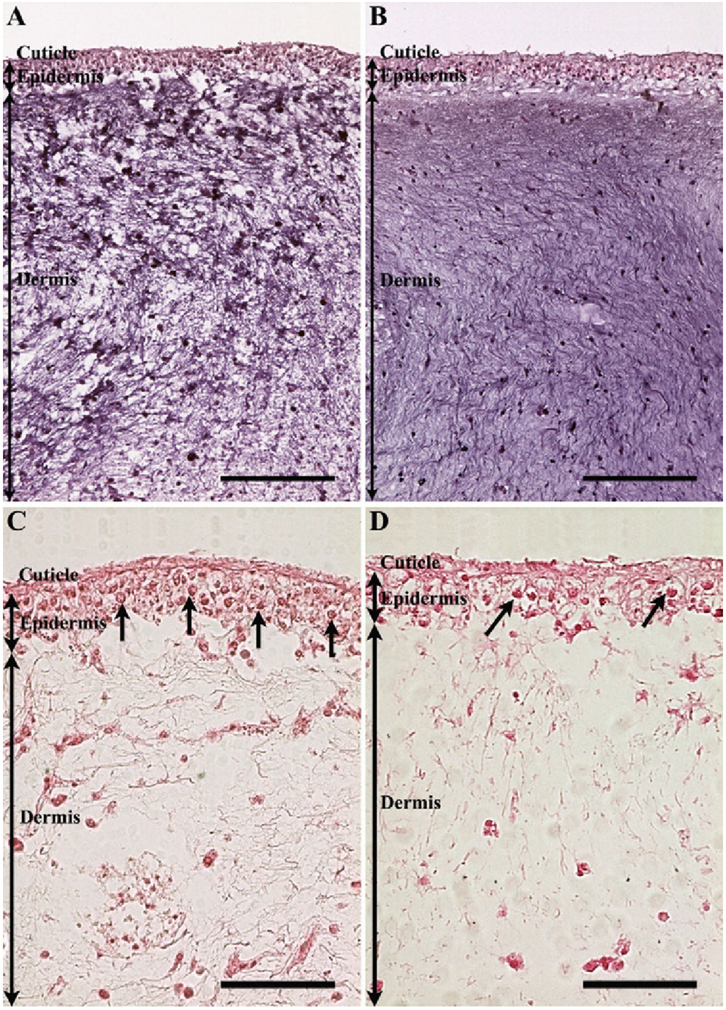

FIGURE 12.6

Histological cross-section of the body wall of normally pigmented (A and C) and albino (B and D) juvenile of *A. japonicus* with melanocytes (arrows) stained with H&E (A and B) and Masson Fontana (C and D). Scale bar (A and B) = 100 μm, (C and D) = 50 μm.

From Zhao, 2011

12.5 DIFFERENCES IN *MITF* GENE EXPRESSIONS IN ALBINO AND NORMAL *A. japonicus*

Colin (2007) has reported that albinism in animals is caused by various inactivations of relevant genes. The tyrosinase (*TYR*) gene family encodes three functional enzymes involved in the melanin biosynthetic pathway, i.e., TYR, tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2). Inactivation of these enzymes can lead to various forms of albinism (William, 2000).

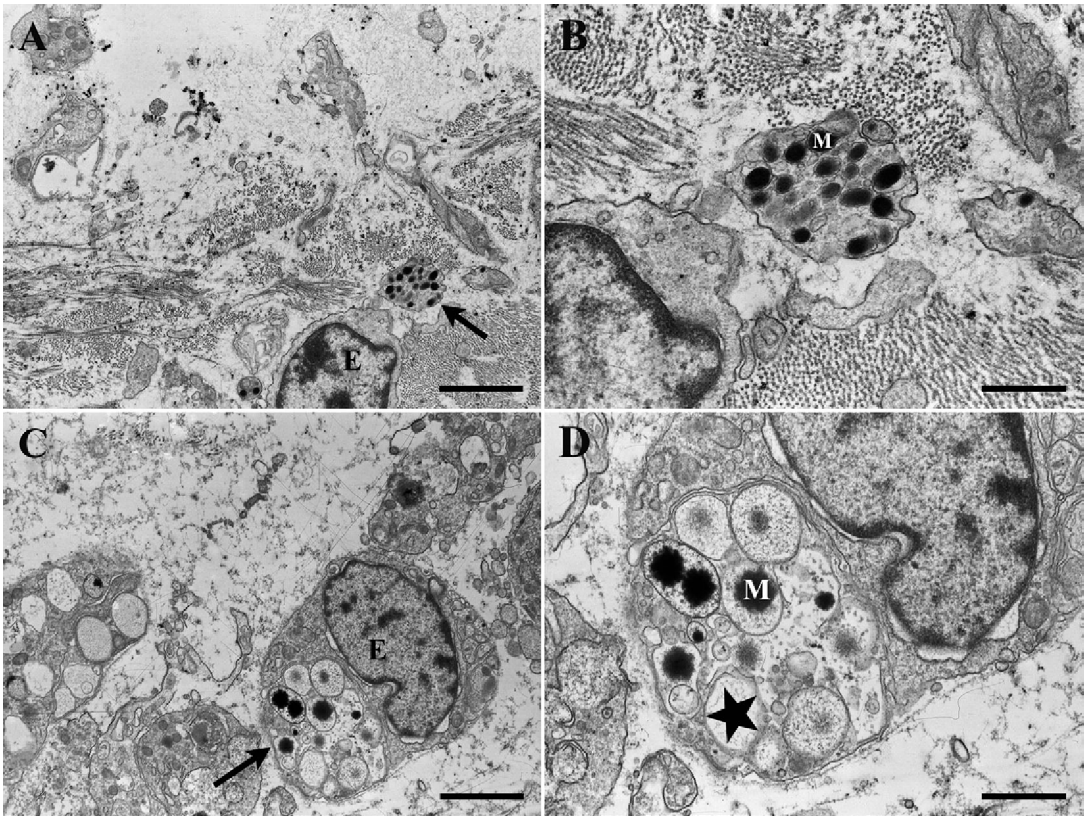

FIGURE 12.7

Electron micrograph of epidermal melanocyte (arrow) of normally pigmented (A) and albino (C) juvenile of *A. japonicus* (M, melanosomes; E, epithelial cell; ★ melanosome vacuoles). Scale bar (A and C) = 0.2 μm, (B and D) = 0.1 μm.

From Zhao, 2011

An important regulator of expression of the *TYR* gene family is the microphthalmia-associated transcription factor (*MITF*). Nakayama et al. (1998) indicated that *MITF* gene expression is essential for melanocyte development in mice, and mutations in *MITF* are responsible for abnormalities in neuroepithelial and neural-crest-derived melanocytes. Furthermore, mutations in the human *MITF* gene are associated with albinism-deafness (Tietz) syndrome (Amiel et al., 1998) and Waardenburg syndrome type 2 (WS2) (Tassabehji et al., 1994, 1995). This latter condition is characterized by varying degrees of deafness, minor defects in structures arising from the neural crest, and pigmentation anomalies. Despite its importance, little is known about the role of the *MITF* gene in *A. japonicus*.

To evaluate which gene might be associated with albinism in *A. japonicus*, the transcriptome of normal and albino *A. japonicus* was sequenced using 454 high-throughput technology. Sequencing analysis revealed that the frequency distribution of the *MITF* contig in normal *A. japonicus* transcriptome contig library was several times higher than for albino *A. japonicus*, suggesting that mRNA expression of the *MITF* gene is lower in albinos. To investigate the correlation between *MITF* gene

expression and albinism in *A. japonicus*, Zhao (2011) cloned the *MITF* full-length cDNA and quantified the differences in *MITF* mRNA expression in the adult body wall and juvenile offspring between albino and normal individuals (see Section 12.5.1).

12.5.1 ISOLATION AND CHARACTERIZATION OF *A. japonicus MITF* FULL-LENGTH cDNA

The full-length cDNA of *A. japonicus MITF* was found to contain 3104 nucleotides. It consisted of a 3′untranslated region (UTR) of 1021 bp, a 5′UTR of 586 bp, and an open-reading frame (ORF) of 1497 nucleotides (position 587–2083). The cDNA sequence of the *MITF* gene was deposited in the NCBI/GenBank under accession number HQ606465. No evidence of nucleotide mutation was found in the cDNA of albino *A. japonicus MITF* (Zhao et al., 2012a).

12.5.2 SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSIS OF *A. japonicus MITF* PROTEIN

The translated *MITF* protein of *A. japonicus* contained 499 amino acids, including a basic helix loop helix (bHLH) domain located at amino acids 279–340. Protein alignments with all known animal *MITF*s revealed that the *A. japonicus MITF* bHLH domain shared 67% similarity with the *Ciona intestinalis MITF* bHLH domain; 74% with the *Drosophila melanogaster MITF* bHLH domain; 75% with the *Saccoglossus kowalevskii MITF* bHLH domain; 82% with the *MITF* bHLH domains of *Mus musculus*, *Rattus norvegicus*, *Homo sapiens*, *Canis lupus familiaris*, *Sus scrofa*, *Mesocricetus auratus*, *Macaca mulatta*, *Equus caballus*, *Gallus gallus*, *Xenopus laevis*, *X.* (*Silurana*) *tropicalis*, and *Danio rerio*; and 83% similarity with the *Poecilia reticulata MITF* bHLH domain. Comparison of *A. japonicus* with other animals confirmed that the bHLH domain is a highly conserved domain. Furthermore, the *A. japonicus MITF* bHLH domain was closely related to vertebrate *MITF* bHLH domains (Figure 12.8). Mutations of *MITF* in albino humans or mice frequently exhibit a compromised region encoding the basic helix loop helix leucine zipper (bHLHZip) domain (Kazuhisa et al., 2000). However, no evidence of nucleotide mutation was detected in the homologous domain of albino *A. japonicus MITF* (Zhao et al., 2012a).

12.5.3 QUANTITATIVE ANALYSIS OF *MITF* mRNA EXPRESSION IN ALBINO AND NORMAL *A. japonicus*

The analysis of *A. japonicus MITF* mRNA expression suggested that the level of expression was lower in the body wall of albino adults (Figure 12.9; Zhao et al., 2012a), which contained fewer epidermal melanocytes than normal adults. This was consistent with the 454 sequencing analysis. Because *MITF* plays an important role in the survival and proliferation of melanocytes in adult humans (Levy et al., 2006), it is reasonable to conclude that the reduced number of epidermal melanocytes in albino *A. japonicus* is due to the decreased expression of *MITF*. Furthermore, in the melanocytes of albino adults, the melanosomes contained less melanin as well as nonpigmented vacuoles. According to Wasmeier et al. (2008), nonpigmented melanosome vacuoles represent premelanosomes in which melanins are not yet deposited. *MITF* regulates the expression of the tyrosinase (*TYR*) gene family through a CATGTG promoter sequence (Jiri and Jan, 2010). Therefore, in adult albino *A. japonicus*, the

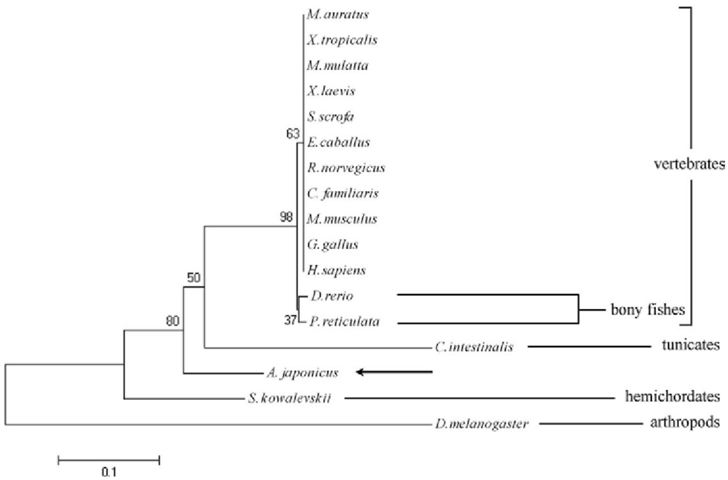

FIGURE 12.8

Phylogenetic analysis of *MITF* in *A. japonicus* and other animals. The arrow denotes *A. japonicus MITF*.

From Zhao, 2011

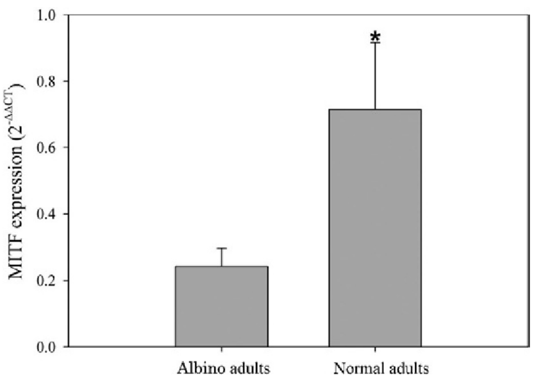

FIGURE 12.9

Relative mRNA abundance of *MITF* in the body wall of albino and normally pigmented adults of *A. japonicus*. The asterisk indicates significant differences ($P < 0.05$) between albino and normal individuals. Values shown as mean ± standard deviation ($n = 5$).

From Zhao, 2011

decrease in expression of the *MITF* gene likely downregulated the expression of the *TYR* gene family, leading to a reduction in the ability of albino individuals to synthesize melanin.

The early juvenile stage is the critical period for melanogenesis. The analysis of *A. japonicus MITF* mRNA expression suggested that the level of expression in normal juveniles increased during the 32 days following fertilization and peaked 46 days postfertilization. Thereafter, *MITF* expression levels decreased steadily before stabilizing. During the same developmental period, the levels of *MITF*

expression rose steadily in juvenile albinos. Compared with normal juveniles, the *MITF* expression levels in albino juveniles were lower 32 days postfertilization (Figure 12.10; Zhao et al., 2012a). By 88 days postfertilization, fewer melanocytes were observed in the epidermis of these albinos. *MITF* is essential for the development of embryonic melanocytes in mice (Opdecamp et al., 1997). The slow development of melanocytes during the early stages of melanogenesis in albino juveniles of *A. japonicus* is likely associated with the decrease in *MITF* expression. In addition, electron microscopy observations of albino juveniles revealed that their melanocytes contained less developed melanosomes than those in normal juveniles. Very little deposition of melanin in the melanosomes of albino juveniles was observed (Zhao et al., 2012a). Because the development and melanization of melanocytes is dependent on activation of the *MITF* gene (Ichiro et al., 2003), the lack of melanocytes and melanin in albino juveniles is likely caused by lower *MITF* expression from an early stage of melanogenesis.

There are many signaling molecules involved in the regulation of *MITF* expression. For example, α-MSH (α-melanocyte stimulating hormone) upregulates the transcriptional activity of *MITF* via a cAMP-dependent pathway whereas ASP (agouti signal protein) downregulates the *MITF* expression and inhibits melanoblast differentiation (Aberdam et al., 1998). Similarly, *WNT* (wingless in *Drosophila*, Int in mouse) is an essential signaling molecule that regulates the derivation of melanocytes from neural crest cells and can induce *MITF* expression (Takeda et al., 2000). Previous 454 sequencing analysis suggested that the frequency distribution of the *WNT* contig was higher in the normal *A. japonicus* transcriptome contig library than in that of albino *A. japonicus*, suggesting that *WNT* mRNA expression is lower in albino individuals. Therefore, the downregulation of *MITF* in albino *A. japonicus* is likely caused by the decreased expression of the *WNT* gene. However, the regulatory

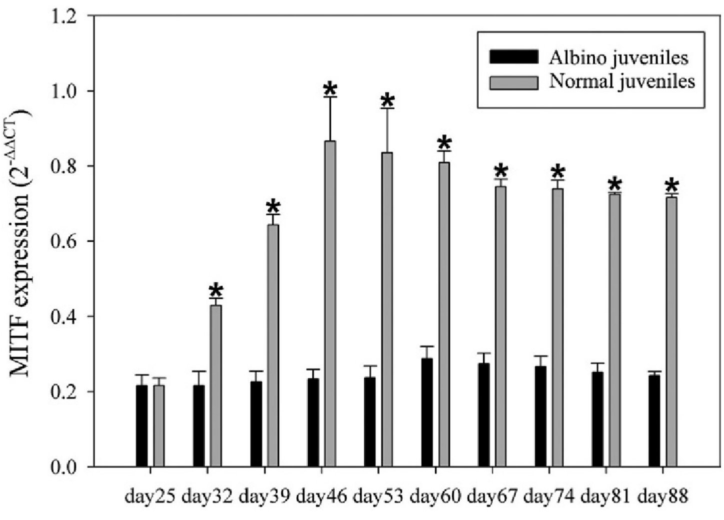

FIGURE 12.10

Relative mRNA abundance of *MITF* in *A. japonicus* juveniles at various stages of development. The asterisk indicates significant differences ($P < 0.05$) between albino and normal individuals. Values shown as mean ± standard deviation ($n = 5$).

From Zhao, 2011

pathways for *MITF* gene expression remain poorly understood in *A. japonicus*, and so deserve further attention.

12.6 DIFFERENCES IN *ASTACIN* GENE EXPRESSIONS IN ALBINO AND NORMAL *A. japonicus*

Astacin is the main carotenoid in marine crustaceans and fish (Leng and Li, 2006). Many research results indicate that adding a moderate amount of *astacin* in feed can improve the coloration of skin and muscle in aquatic organisms and consequently improve their ornamental and commercial values (Song et al., 2007). The *astacin* family is a novel family of Zn-metalloendopeptidases distributed in developmental systems of vertebrates and invertebrates (Bond and Beynon, 1995). The *astacin* family proteases, synthesized with NH2-terminal signal and proenzyme sequences, share similar amino acid sequence, functional structure, and substrate specificity. The signature sequence of the *astacin* family is HEXXHXXGFXHEXXRXDRD (Bode et al., 1992, 1993).

The 454 sequencing analysis revealed that the frequency distribution of the *astacin* contig in the normal *A. japonicus* transcriptome contig library was several times higher than for the albino individuals, suggesting that mRNA expression of the *astacin* gene is lower in albino *A. japonicus* (Zhao, 2011). To investigate the correlation between *astacin* gene expression and albinism in *A. japonicus*, Zhao (2011) cloned the *astacin* full-length cDNA and quantified the differences in *astacin* mRNA expression in the adult body wall and juvenile offspring between albino and normal individuals (see Section 12.6.1).

12.6.1 ISOLATION AND CHARACTERIZATION OF *ASTACIN* FULL-LENGTH cDNA

The full-length cDNA of *A. japonicus astacin* contained 2058 nucleotides. It consisted of a 3′untranslated region (UTR) of 187 bp, a 5′UTR of 188 bp, and an open-reading frame (ORF) of 1683 nucleotides (position 188–1870). The cDNA sequence of the *astacin* gene was deposited in the NCBI/GenBank under accession number HQ655812. No evidence of nucleotide mutation in the cDNA of albino *A. japonicus astacin* was found. The translated *A. japonicus astacin* protein contained 560 amino acids, including a Zn-metalloendopeptidases functional domain located at amino acids 107–248. This functional domain contained a highly conserved HEXXHXXGFXHEXXRXDRD sequence with HEXXH zinc-binding motif (Zhao et al., 2012b).

12.6.2 SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSIS OF *ASTACIN* PROTEIN

Protein alignments with all known animal *astacin*s revealed that the *A. japonicus astacin* Zn-metalloendopeptidases functional domain shared high similarity with that of vertebrates, especially in the HEXXHXXGFXHEXXRXDRD sequence. Comparison of *A. japonicus* with other animals confirmed that HEXXHXXGFXHEXXRXDRD sequences are highly conserved between vertebrates and invertebrates. Furthermore, the *A. japonicus astacin* Zn-metalloendopeptidases functional domain was closely related to that of *Strongylocentrotus purpuratus* (Figure 12.11; Zhao et al., 2012b).

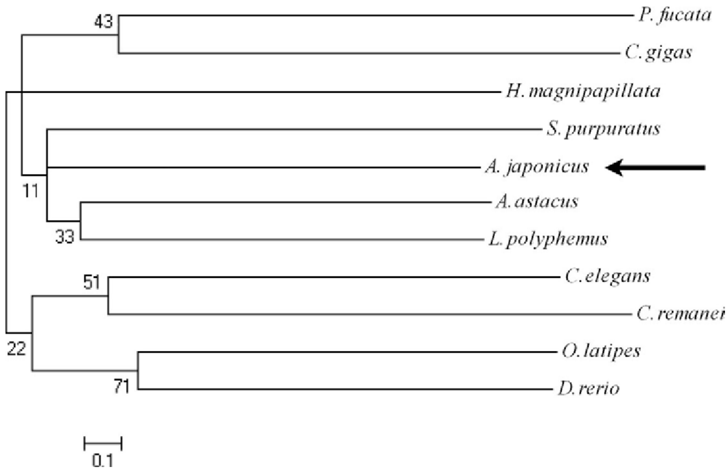

FIGURE 12.11

Phylogenetic analysis of *astacin* in *A. japonicus* and other animals. The arrow denotes *A. japonicus astacin*.

From Zhao, 2011

12.6.3 QUANTITATIVE ANALYSIS OF *ASTACIN* mRNA EXPRESSION

Astacin is an important carotenoid in the coloration of aquatic organisms, which was first discovered in crayfish hepatopancreas (Vogt et al., 1989). Research indicates that lutein and zeaxanthin can transform into *astacin* in fish; and β-carotene, lutein, and zeaxanthin can transform into *astacin* in most crustaceans (Huang, 2008). Yamaguchi and Miki (1981) reported that the carotenoid content in the skin of albino rainbow trout is lower than in normal rainbow trout. Furthermore, in aquatic organisms, adding carotenoid or yeast, and algae rich in *astacin* can improve the integument coloration (Zhang et al., 2008). The analysis of *A. japonicus astacin* mRNA expression suggested that the level of expression was lower in the body wall of adult albinos (Figure 12.12; Zhao et al., 2012b), which have fewer pigments than normal adults. This was consistent with the 454 sequencing analysis.

During the process of melanogenesis, *astacin* mRNA expression in normal juveniles increased during the 39 days following fertilization and peaked 53 days postfertilization (Zhao et al., 2012b). Thereafter, *astacin* expression levels decreased gradually before stabilizing. During the same developmental period, the levels of *astacin* expression rose steadily, but not significantly, in juvenile albinos. Compared with normal juveniles, the *astacin* expression levels in juvenile albinos were lower at 39 days postfertilization (Figure 12.13; Zhao et al., 2012b). The depigmentation of integument during the early stages in albino *A. japonicus* juveniles is likely associated with the decrease in *astacin* expression.

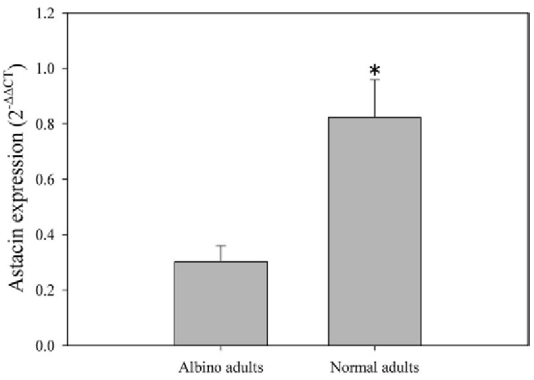

FIGURE 12.12

Relative mRNA abundance of *astacin* in the body wall of albino and normal adults of *A. japonicus*. The asterisk indicates significant differences ($P < 0.05$) between albino and normal individuals. Values shown as mean ± standard deviation ($n = 5$).

From Zhao, 2011

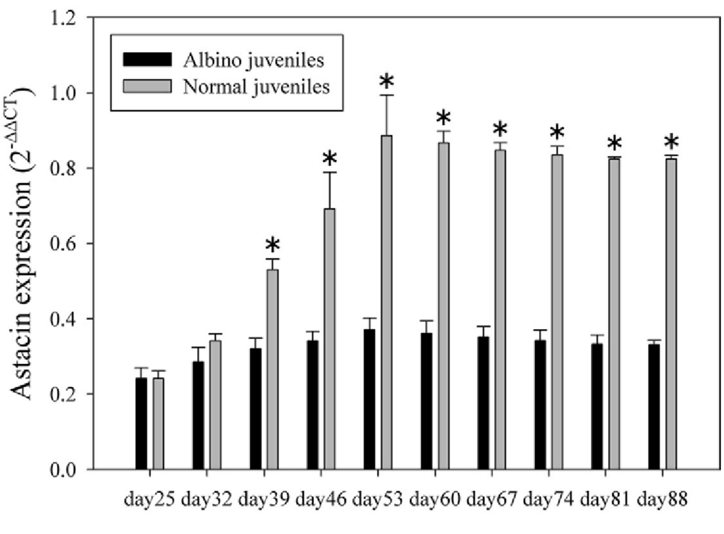

FIGURE 12.13

Relative mRNA abundance of *astacin* in juveniles of *A. japonicus* at various stages of development. The asterisk indicates significant differences ($P < 0.05$) between albino and normal individuals. Values shown as mean ± standard deviation ($n = 5$).

From Zhao, 2011

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CHAPTER

TISSUE BIOCHEMISTRY

13

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SUMMARY

The sea cucumber *Apostichopus japonicus* has a long history of use as a traditional tonic consumed by the Chinese and Japanese for its nutritional value as well as medicinal properties. Most of the edible and medicinal properties are derived from the body wall tissue (in China) and digestive tract and gonad (in Japan). In this chapter, the proximate composition (moisture, crude protein, carbohydrate, lipid, and ash content), amino acid, fatty acid, vitamins, and minerals in the different tissues of the sea cucumber *A. japonicus* are outlined.

Keywords: amino acids; *Apostichopus japonicus*; biochemistry; fatty acids; minerals; proximate composition; sea cucumber; vitamins

13.1 PROXIMATE COMPOSITION

13.1.1 IN THE BODY WALL AND VISCERA

The water content in the body wall and viscera of *Apostichopus japonicus* was found to be high (from 82.6 to 91.9%, Gao et al., 2011; Yuan et al., 2010; Tables 13.1 and 13.2), as had been observed in other sea cucumbers (Svetashev et al., 1991). More precisely, the water content in the digestive tract (82.63±0.81%) and respiratory tree (84.93±0.76%) were both lower than in the body wall tissues (91.20±1.13%) of *A. japonicus* (Yuan et al., 2010; Table 13.1), while the water content in the body wall was similar to that of viscera in previously studied species from tropical and temperate zones (Yuan et al., 2010; Svetashev et al., 1991). In addition, the lipid content in viscera was about three times (1.93±0.15% in digestive tract, 1.50±0.10% in respiratory tree) as rich as in the body wall (0.57±0.03%; Yuan et al., 2010; Table 13.1), which was consistent with previous data in other sea cucumbers (Svetashev et al., 1991). In *A. japonicus*, the protein contents in the digestive tract and respiratory tree were also higher than those in the body wall. Inversely, ash content in the body wall (3.55±0.29%) was higher than that in the viscera (2.87±0.08% in digestive tract and 2.60±0.07% in respiratory tree; Yuan et al., 2010).

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Table 13.1 Proximate Composition of Fresh *A. japonicus* Body Wall, Digestive Tract, and Respiratory Tree

Organ	Water	Ash	Lipid	Protein
Body wall	91.20 ± 1.13	3.55 ± 0.29	0.57 ± 0.03	3.53 ± 0.12
Digestive tract	82.63 ± 0.81	2.87 ± 0.08	1.93 ± 0.15	11.36 ± 0.31
Respiratory tree	84.93 ± 0.76	2.60 ± 0.07	1.50 ± 0.10	8.94 ± 0.19

The sea cucumbers were collected from Shandong Homey Aquatic Development Co. LTD (China). Values are presented as mean ± SD (in % of wet weight).
From Yuan et al., 2010

Table 13.2 Seasonal Variation in Proximate Composition of the Body Wall of Fresh *A. japonicus*

Date		Water	Ash	Carbohydrate	Lipid	Protein
Jul. 20, 2006	WET	91.10 ± 1.21[a]	3.11 ± 0.07[a]	0.45 ± 0.10[ab]	0.24 ± 0.08[bcd]	3.77 ± 0.29[bc]
	DRY		36.94 ± 2.13[abc]	5.34 ± 0.74[a]	2.85 ± 0.80[bc]	44.69 ± 2.55[b]
Aug. 6, 2006	WET	90.21 ± 0.58[ab]	3.30 ± 0.05[a]	0.41 ± 0.09[abc]	0.31 ± 0.03[abc]	4.37 ± 0.38[b]
	DRY		34.12 ± 2.42[abc]	4.27 ± 0.72[ab]	3.25 ± 0.32[ab]	45.22 ± 1.71[b]
Sep. 5, 2006	WET	89.05 ± 0.64[b]	3.25 ± 0.05[a]	0.55 ± 0.07[a]	0.37 ± 0.10[ab]	5.40 ± 0.46[a]
	DRY		31.06 ± 0.75[c]	5.03 ± 0.29[a]	3.44 ± 0.92[ab]	49.83 ± 1.14[a]
Oct. 10, 2006	WET	90.80 ± 0.35[ab]	2.99 ± 0.37[a]	0.40 ± 0.06[abc]	0.15 ± 0.06[cd]	4.35 ± 0.23[b]
	DRY		31.44 ± 4.81[bc]	4.17 ± 0.42[ab]	1.53 ± 0.62[c]	45.60 ± 0.54[b]
Nov.27, 2006	WET	91.90 ± 0.52[a]	3.23 ± 0.10[a]	0.25 ± 0.06[c]	0.12 ± 0.02[d]	3.51 ± 0.43[c]
	DRY		39.64 ± 1.65[a]	3.08 ± 0.48[b]	1.44 ± 0.27[c]	42.87 ± 2.24[b]
Jan. 24, 2007	WET	91.12 ± 0.84[ab]	3.33 ± 0.28[a]	0.36 ± 0.05[bc]	0.15 ± 0.01[cd]	3.97 ± 0.14[bc]
	DRY		37.68 ± 2.74[abc]	4.05 ± 0.49[ab]	1.64 ± 0.11[c]	44.88 ± 0.87[b]
Mar. 29, 2007	WET	90.92 ± 1.18[ab]	3.30 ± 0.08[a]	0.46 ± 0.01[ab]	0.41 ± 0.03[a]	3.66 ± 0.06[bc]
	DRY		37.84 ± 1.22[ab]	5.31 ± 0.16[a]	4.65 ± 0.37[a]	41.90 ± 0.55[b]
Jun. 11, 2007	WET	90.77 ± 0.26[ab]	3.26 ± 0.06[a]	0.44 ± 0.08[ab]	0.28 ± 0.03[abc]	3.92 ± 0.05[bc]
	DRY		37.44 ± 1.69[ab]	5.02 ± 0.75[a]	3.16 ± 030[b]	45.01 ± 1.43[b]

The sea cucumbers were collected from a farm in Qingdao along the coast of the Yellow Sea (China). WET, proportion of wet weight (%); DRY, proportion of dry weight (%). Values are presented as mean ± SD. Different letters show significant differences among the sampling dates ($P < 0.05$).
From Gao et al., 2011

13.1.2 SEASONAL VARIATION IN PROXIMATE COMPOSITION OF BODY WALL

The biochemical components of marine organisms vary with seasonal changes in water temperature and food availability (Dunstan et al., 1999; David and MacDonald, 2002). The seasonal biochemical composition of the body wall of the sea cucumber *A. japonicus* is no exception.

Water content of body wall tissues in *A. japonicus* clearly fluctuate across seasons, from 89 to 93% (Gao et al., 2011; Li et al., 2006; Table 13.2). In the sea cucumbers sampled from a farm in Qingdao along the coast of the Yellow Sea (China), the minimum moisture content occurred in September, while most of the sea cucumbers were in deep aestivation (Gao et al., 2011) (see Chapter 11 for details on aestivation). The moisture content increased in autumn and reached maximum values in November (Gao et al., 2011). However, in *A. japonicus* sampled near Hakodate (Japan), the water content in the body wall showed a maximum value (91%) in July, and decreased to a minimum (85%) in January of the following year (Tanikawa et al., 1955).

Ash content in fresh body wall of *A. japonicus* sampled from farmed areas in the Yellow Sea in China varied from 2.6 to 3.3% across seasons (Gao et al., 2011; Li et al., 2006, Table 13.2). Ash content in sea cucumbers is higher than in other sea foods, such as scallops, shrimp, and cephalopods (Yanar and Celik, 2006; Wang, 2007; Ozogul et al., 2008), due to the many calcareous ossicles found in body wall tissues.

Protein content in the fresh body wall of *A. japonicus* also varied with seasons (3.5–5.4%, Table 13.2). The highest values were reached in September, while most sea cucumbers were in aestivation (Gao et al., 2011).

Lipid content in the fresh body wall tissues of *A. japonicus* was 0.1–0.6% across seasons. The peak lipid content occurred in March; it was higher than contents in October, November, and January (Table 13.2; Gao et al., 2011; Li et al., 2006; Kasai, 2003).

In the fresh body wall of *A. japonicus* sampled from a farm in Qingdao, total carbohydrate content varied seasonally (0.3–0.6%), with maximum and minimum values recorded in September and November, respectively (Table 13.2; Gao et al., 2011).

Protein and ash were the major components of the body wall tissues in *A. japonicus*, clearly higher than carbohydrate and lipid contents (in dry weight; Gao et al., 2011; Li et al., 2006). This result is consistent with data from the temperate North Atlantic sea cucumber, *Cucumaria frondosa* (Zhong et al., 2007). However, in some other species, such as *Actinopyga agassizi*, *Synaptula hydriformis*, *Pentacta pygmaea*, and *Holothuria leucospilota*, both lipids and proteins are important components compared to carbohydrates (Prim et al., 1976; Jaya Sree et al., 1994). During the aestivation period, *A. japonicus* ceases to feed. Interestingly, crude protein, carbohydrate, and lipid contents of fresh body wall tissues were comparatively high when the moisture content was at its minimum. As *A. japonicus* loses 30–50% of its body weight during aestivation, it can be suggested that the main nutritional constituents of the body wall, including protein, carbohydrate, and lipid, may all participate in the energy supply. The results may also show that there are no special needs for lipid as a source of energy during the aestivation period. The digestive tract of some species of sea cucumbers, such as *Trachythyone elongata*, *C. frondosa*, and *H. scabra*, is considered an organ for energy storage (Hamel and Mercier, 1996; Jangoux and Lawrence, 1982). *A. japonicus* decreases the width of its digestive tracts to approximately 1 mm in diameter and shortens it to about half of its original length during aestivation (Liu et al., 1996). Therefore, the digestive tract may also supply energy while the sea cucumber gradually enters the aestivation state, which could explain the seasonal fluctuations in its chemical composition.

13.2 AMINO ACIDS

A total of 18 amino acids were identified in the dry body wall tissues, digestive tract, and respiratory tree of *A. japonicus*. They were alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine, cysteine, tyrosine, aspartic acid, glutamic acid, lysine, arginine, and histidine (Tables 13.3 and 13.4; Gao et al., 2011; Li et al., 2006; Xiang et al., 2006; Yuan et al., 2010).

The contents of essential amino acids (EAA) in the dry body wall tissues of *A. japonicus* varied from 12 to 14%, and the ratios of EAA to total amino acids (TAA) in body wall tissues were

Table 13.3 Amino Acid Composition in the Body Wall, Digestive Tract, and Respiratory Tree of Fresh *A. japonicus*

Amino Acids	Body Wall	Digestive Tract	Respiratory Tree
Aspartic acid	3.66	4.56	4.18
Threonine	1.93	2.32	2.40
Serine	1.62	2.26	2.01
Glutamic acid	6.13	8.30	8.32
Glycine	3.87	2.92	3.36
Proline	2.57	1.76	1.97
Alanine	2.24	2.63	3.18
Cysteine	0.57	0.53	0.50
Valine	1.60	2.30	2.24
Methionine	0.61	1.00	0.99
Isoleucine	1.37	2.07	2.03
Leucine	1.86	3.20	3.15
Tyrosine	0.96	1.80	1.57
Phenylalanine	1.16	2.18	1.91
Lysine	1.61	3.41	3.73
Histidine	0.22	0.50	0.49
Tryptophan	2.68	0.54	0.57
Arginine	2.36	2.82	2.97
TAA	37.02	45.12	45.58
EAA	12.82	17.04	17.02
EAA/TAA	34.63	37.77	37.74

The sea cucumbers were collected from Shandong Homey Aquatic Development Co. LTD (China). EAA, essential amino acids; NEAA, nonessential amino acids; TAA, total amino acids (China). Values provided as % of dry weight.
From Yuan et al., 2010

Table 13.4 Seasonal Variation in the Amino Acid Profile in Body Wall Tissues of Fresh *A. japonicus*

Amino Acids	Jul. 20, 2006	Aug. 6, 2006	Sep. 5, 2006	Oct. 10, 2006	Nov. 27, 2006	Jan. 24, 2007	Mar. 29, 2007	Jun. 11, 2007
Aspartic acid	3.76 ± 0.15	4.16 ± 0.11	4.35 ± 0.27	4.22 ± 0.02	3.76 ± 0.26	3.92 ± 0.24	3.76 ± 0.11	4.13 ± 0.26
Threonine	1.69 ± 0.08	1.88 ± 0.06	1.93 ± 0.13	1.85 ± 0.01	1.62 ± 0.15	1.66 ± 0.09	1.65 ± 0.04	1.83 ± 0.12
Serine	2.18 ± 0.08	2.39 ± 0.06	2.50 ± 0.15	2.29 ± 0.00	2.04 ± 0.12	2.13 ± 0.13	2.19 ± 0.05	2.41 ± 0.14
Glutamic acid	4.14 ± 0.18	4.56 ± 0.11	4.87 ± 0.32	5.07 ± 0.02	4.66 ± 0.20	5.17 ± 0.34	4.18 ± 0.15	4.58 ± 0.31
Glycine	4.59 ± 0.36	4.71 ± 0.10	5.35 ± 0.38	6.26 ± 0.04	5.74 ± 0.20	6.00 ± 0.41	4.58 ± 0.22	5.14 ± 0.50
Alanine	7.11 ± 0.45	7.45 ± 0.15	8.38 ± 0.60	9.59 ± 0.00	8.90 ± 0.26	9.28 ± 0.68	7.15 ± 0.33	7.94 ± 0.70
Cysteine	0.51 ± 0.03	0.54 ± 0.02	0.58 ± 0.04	0.77 ± 0.01	0.69 ± 0.04	0.67 ± 0.08	0.47 ± 0.00	0.57 ± 0.03
Valine	1.97 ± 0.10	2.21 ± 0.07	2.25 ± 0.16	2.05 ± 0.02	1.84 ± 0.15	1.90 ± 0.08	1.97 ± 0.06	2.15 ± 0.14
Methionine	0.54 ± 0.03	0.61 ± 0.02	0.59 ± 0.05	0.50 ± 0.02	0.43 ± 0.05	0.42 ± 0.00	0.52 ± 0.01	0.55 ± 0.07
Isoleucine	1.34 ± 0.07	1.51 ± 0.06	1.54 ± 0.10	1.44 ± 0.02	1.29 ± 0.11	1.35 ± 0.07	1.34 ± 0.04	1.46 ± 0.10
Leucine	2.03 ± 0.11	2.32 ± 0.10	2.35 ± 0.16	2.32 ± 0.02	2.07 ± 0.23	2.21 ± 0.13	2.05 ± 0.06	2.21 ± 0.15
Tyrosine	0.97 ± 0.05	1.10 ± 0.04	1.12 ± 0.08	1.05 ± 0.03	0.92 ± 0.08	0.96 ± 0.06	0.97 ± 0.03	1.07 ± 0.06
Phenylalanine	1.25 ± 0.07	1.43 ± 0.06	1.42 ± 0.09	1.22 ± 0.02	1.08 ± 0.11	1.12 ± 0.04	1.25 ± 0.03	1.36 ± 0.09
Lysine	1.57 ± 0.10	1.79 ± 0.09	1.83 ± 0.12	1.54 ± 0.01	1.40 ± 0.14	1.53 ± 0.07	1.62 ± 0.08	1.70 ± 0.10
Histidine	0.77 ± 0.03	0.87 ± 0.03	0.87 ± 0.08	—	—	—	0.98 ± 0.35	0.83 ± 0.06
Arginine	2.61 ± 0.14	2.85 ± 0.08	3.12 ± 0.24	3.09 ± 0.06	2.79 ± 0.19	2.98 ± 0.21	2.59 ± 0.07	2.89 ± 0.25
Proline	2.61 ± 0.16	2.78 ± 0.06	3.02 ± 0.22	3.00 ± 0.06	2.80 ± 0.16	2.91 ± 0.17	2.60 ± 0.08	2.93 ± 0.25
TAA	39.62 ± 1.87^{b}	43.16 ± 1.03^{ab}	46.06 ± 3.15^{a}	46.25 ± 0.17^{a}	42.06 ± 2.28^{ab}	44.20 ± 2.74^{ab}	39.87 ± 1.66^{b}	43.74 ± 3.01^{ab}
EAA	12.57 ± 0.60^{bc}	14.14 ± 0.52^{ab}	14.41 ± 0.95^{a}	13.20 ± 0.08^{abc}	11.78 ± 1.06^{c}	12.31 ± 0.62^{bc}	12.59 ± 0.38^{abc}	13.67 ± 0.86^{ab}
EAA/NEAA	0.465 ± 0.022^{a}	0.487 ± 0.012^{a}	0.455 ± 0.003^{a}	0.399 ± 0.002^{b}	0.389 ± 0.022^{b}	0.386 ± 0.006^{b}	0.461 ± 0.008^{a}	0.456 ± 0.027^{a}
EAA/TAA	0.317 ± 0.010^{a}	0.328 ± 0.006^{a}	0.313 ± 0.002^{a}	0.285 ± 0.001^{b}	0.280 ± 0.011^{b}	0.279 ± 0.003^{b}	0.316 ± 0.004^{a}	0.313 ± 0.012^{a}

The sea cucumbers were collected from a farm in Qingdao along the coast of the Yellow Sea (China). Values are presented as mean ± SD in % dry weight.
From Gao et al., 2011

0.28–0.33 across all seasons (Tables 13.3 and 13.4; Gao et al., 2011; Yuan et al., 2010). The EAA contents in the digestive tract (17%) and respiratory tree (17%) were both higher than that in the body wall tissues (Table 13.3; Yuan et al., 2010), and the EAA/TAA ratios in viscera (~37%) were higher than that in the body wall (Gao et al., 2011; Yuan et al., 2010). The study of Gao et al. (2011) showing the minimum EAA/TAA ratios in the body wall of *A. japonicus*, sampled from Qingdao, were observed in January when the water temperature was at its lowest of the year, and then increased with ambient water temperature; the ratios peaked in August while the ambient water temperature was at its highest, and then dropped with decreasing ambient water temperature. The ratios of EAA to nonessential amino acids (NEAA) were 0.39–0.49 in different seasons (Table 13.4; Gao et al., 2011), which is lower than those in some shrimps, crabs, squids, and fishes (Iwasaki and Harada, 1985; Yanar and Celik, 2006).

13.3 FATTY ACIDS

13.3.1 MAJOR FATTY ACIDS IN DIFFERENT TISSUES

In the dry body wall tissues of *A. japonicus* sampled from Qingdao, the major fatty acids (≥5% of the total fatty acids) were 16:0, 18:0, 16:1n-7, 18:1n-7, 20:4n-6, and 20:5n-3 in all seasons (Gao et al., 2011; Table 13.5). These fatty acids were also the major fatty acids in *A. japonicus* collected in the Pacific Ocean along the Mori Coast of Southern Hokkaido, Japan (Kaneniwa et al., 1986; Table 13.6). However, Kaneniwa et al. (1986) indicated that the major fatty acids in *A. japonicus* caught in the Pacific Ocean off the Shikabe Coast in Southern Hokkaido were 16:0, 16:1n-7, 18:0, 18:1n-7, and 20:1n-9 (Table 13.6). The fatty acids *iso*-C_{15}, 16:0, 16:1n-7, 18:0, 18:1n-7, 20:4n-6, 20:5n-3, 23:1n-9, and 22:6n-3 were the principal fatty acids in the body wall of the male and female sea cucumbers collected in the Sea of Okhotsk off the shore of Abashiri, Japan (Kasai, 2003; Table 13.6). Sun et al. (2010) also reported the different major fatty acids (16:0, 16:1n-7, 16:3n3, 18:0, 20:4n-6, 20:5n-3) in *A. japonicus* collected from a farm in northern China (Dalian Bangchuidao Seafood Enterprise Group Co., Ltd; Table 13.6). Kasai (2003) reported that the digestive tract of male and female *A. japonicus* collected in the Sea of Okhotsk (Japan) had a comparable composition in major fatty acids (Table 13.6).

Like the digestive tract, the gonad of male and female *A. japonicus* collected from the same sea area of Okhotsk (Japan), showed identical major fatty acids composition and similar contents for each fatty acid (Table 13.4; Kasai, 2003). However, the composition and contents of the major fatty acids in gonads were different among sea cucumbers sampled from different sites (Table 13.6; Kasai, 2003; Sun et al., 2010).

13.3.2 SEASONAL VARIATION OF FATTY ACIDS IN BODY WALL TISSUES

Gao et al. (2011) studied the seasonal variation of fatty acids in body wall tissues of *A. japonicus* collected from Qingdao in China. They found a total of 39 fatty acids, including eight saturated fatty acids (SFA), 11 monounsaturated fatty acids (MUFA), and 18 polyunsaturated fatty acids (PUFA; Table 13.6).

The proportions of SFA in the body wall varied seasonally (from 17% in January to 31% in August; Gao et al., 2011). The proportions of MUFA showed no seasonal variation, remaining around 30–35% across months. PUFA showed the opposite pattern to SFA, as the maximum of 43% and minimum of 26% were recorded in January and August, respectively (Gao et al., 2011). The variation of SFA and

Table 13.5 Seasonal Variation of Fatty Acid Composition in Fresh *A. japonicus*

Fatty Acids	Jul. 20, 2006	Aug. 6, 2006	Sep. 5, 2006	Oct. 10, 2006	Nov. 27, 2006	Jan. 24, 2007	Mar. 29, 2007	Jun. 11, 2007
SFA								
14:0	1.59±0.27	2.01±0.32	1.76±0.10	1.37±0.07	0.98±0.08	0.88±0.26	1.62±0.09	1.88±0.31
15:0	0.38±0.08	0.56±0.12	0.45±0.06	0.34±0.07	0.24±0.02	0.18±0.04	0.20±0.01	0.29±0.10
16:0	14.64±0.58	16.33±1.47	14.90±0.85	11.90±1.25	11.57±0.72	7.87±0.34	10.95±0.13	12.47±0.74
17:0	1.24±0.16	1.51±0.24	1.39±0.09	1.18±0.21	1.05±0.07	0.56±0.03	0.71±0.02	0.98±0.07
18:0	7.29±0.28	7.71±1.28	7.47±0.93	6.79±0.57	5.63±0.40	4.88±0.23	6.55±0.43	7.20±0.97
20:0	1.31±0.08	1.07±0.55	1.41±0.10	1.50±0.05	1.30±0.08	0.85±0.45	0.85±0.09	1.32±0.09
21:0	0.65±0.08	0.70±0.10	0.84±0.07	0.93±0.17	0.87±0.04	0.97±0.11	0.43±0.04	0.61±0.13
22:0	0.69±0.39	1.01±0.13	0.99±0.15	1.01±0.17	0.95±0.01	0.92±0.05	0.59±0.08	0.86±0.16
MUFA								
14:1 n-5	1.06±0.16	1.10±0.18	1.18±0.14	1.01±0.15	0.94±0.09	0.65±0.13	0.89±0.23	1.24±0.14
16:1 n-9	1.54±0.10	2.34±1.29	1.79±0.13	1.64±0.13	1.51±0.07	1.05±0.05	0.91±0.05	1.25±0.13
16:1 n-7	12.84±1.60	12.19±1.94	10.84±0.24	10.69±2.18	12.15±1.17	14.30±1.31	17.35±6.54	13.05±1.12
16:1 n-5	1.38±0.12	1.23±0.12	1.24±0.22	1.09±0.13	1.34±0.25	0.73±0.02	0.88±0.09	1.08±0.05
17:1 n-9	0.78±0.07	0.77±0.09	0.68±0.04	0.73±0.11	0.70±0.07	1.02±0.10	1.07±0.02	0.75±0.04
18:1 n-9	2.85±0.29	2.55±0.39	2.54±0.08	2.35±0.38	2.61±0.09	2.32±0.21	2.73±0.35	3.45±0.59
18:1 n-7	6.60±0.22	6.44±0.49	5.85±0.19	6.40±0.19	6.79±0.31	6.86±0.10	6.16±0.16	5.92±0.38
20:1 n-11	2.14±0.14	2.10±0.14	2.58±0.11	3.06±0.42	2.91±0.32	2.42±0.25	1.44±0.06	2.26±0.22
20:1 n-9	2.74±0.41	2.43±0.46	2.34±0.10	2.26±0.34	2.59±0.21	2.52±0.16	3.20±0.39	2.62±1.36
22:1 n-9	0.74±0.14	0.66±0.06	0.74±0.06	2.27±0.05	1.07±0.07	0.97±0.05	0.78±0.03	0.87±0.05
20:1 n-7	0.14±0.01	0.15±0.02	0.16±0.03	2.28±0.03	—	0.15±0.01	0.11±0.00	0.11±0.03
PUFA								
16:2 n-4	0.52±0.21	0.28±0.04	0.378±0.02	0.21±0.03	0.24±0.05	0.15±0.01	0.16±0.06	0.34±0.06
16:3 n-3	1.35±0.70	1.70±0.39	1.58±0.29	2.56±0.46	1.91±1.70	3.16±1.30	1.44±0.07	1.83±0.71
16:4 n-3	0.22±0.05	0.28±0.03	0.26±00.05	0.33±0.02	0.36±0.09	0.50±0.18	0.32±0.03	0.29±0.05

(Continued)

Table 13.5 Seasonal Variation of Fatty Acid Composition in Fresh *A. japonicus* (cont.)

Fatty Acids	Jul. 20, 2006	Aug. 6, 2006	Sep. 5, 2006	Oct. 10, 2006	Nov. 27, 2006	Jan. 24, 2007	Mar. 29, 2007	Jun. 11, 2007
18:2 n-6	1.27±0.10	1.23±0.16	1.17±0.17	1.09±0.08	1.01±0.04	0.86±0.06	1.40±0.14	1.68±0.17
18:3 n-6	0.51±0.07	0.49±0.11	0.52±0.07	0.49±0.09	0.39±0.07	0.62±0.03	0.87±0.08	0.56±0.09
18:3 n-3	0.86±0.27	0.63±0.11	0.70±0.06	0.66±0.04	0.66±0.05	0.64±0.02	0.61±0.04	0.60±0.07
18:4 n-3	0.60±0.05	0.62±0.12	0.65±0.07	0.82±0.27	0.67±0.08	0.79±0.04	0.96±0.15	1.06±0.22
20:2 n-6	0.72±0.03	0.56±0.11	0.71±0.06	0.82±0.09	0.75±0.06	0.73±0.13	0.62±0.04	0.72±0.04
20:3 n-6	0.33±0.02	0.29±0.03	0.31±0.01	0.34±0.02	0.31±0.01	0.31±0.00	0.32±0.01	0.33±0.02
20:3 n-3	1.13±0.06	0.99±0.07	1.27±0.06	1.50±0.20	1.47±0.04	1.61±0.09	0.94±0.05	0.89±0.13
20:4 n-6	5.78±0.36	4.88±0.71	6.49±0.10	7.49±1.17	7.29±0.14	8.16±0.68	4.54±0.18	6.44±0.82
20:4 n-3	1.68±0.25	1.58±0.18	2.58±0.30	2.72±0.45	2.25±0.29	2.55±0.68	1.24±0.09	2.06±0.36
20:5 n-3	8.02±0.83	7.34±1.38	7.24±0.91	8.84±1.17	9.49±1.12	14.45±0.32	11.83±0.90	8.26±0.34
22:3 n-9	1.29±0.10	1.25±0.08	1.37±0.16	1.68±0.06	1.93±0.05	2.03±0.18	1.33±0.16	1.29±0.09
22:3 n-6	0.24±0.02	0.23±0.04	0.21±0.03	0.26±0.05	0.25±0.02	0.45±0.03	0.39±0.04	0.28±0.02
22:4 n-6	0.67±0.04	0.57±0.09	0.69±0.01	0.88±0.12	0.86±0.02	0.88±0.06	0.63±0.04	1.06±0.11
22:5 n-3	0.61±0.10	0.53±0.12	0.56±0.005	0.70±0.12	0.73±0.03	0.60±0.02	0.49±0.03	0.58±0.05
22:6 n-3	2.66±0.36	2.42±0.47	2.41±0.11	3.04±0.19	3.76±0.03	4.45±0.20	3.39±0.13	3.15±1.23
BFA								
14-isobr	2.70±0.24	3.04±0.34	3.14±0.29	2.74±0.12	2.58±0.58	1.80±0.33	1.92±0.28	2.37±0.45
14-anteiso	1.49±0.015	1.71±0.17	1.73±0.17	1.49±0.12	1.49±0.04	1.14±0.10	0.98±0.012	1.39±0.18
ΣSFA	27.80±0.47^{ab}	30.89±3.79^{a}	29.21±1.83^{ab}	25.03±1.72^{bc}	22.58±0.76^{c}	17.11±0.32^{d}	21.90±0.61^{cd}	25.62±1.44^{bc}
ΣMUFA	32.80±2.09^{a}	31.96±1.63^{a}	29.94±0.37^{a}	30.32±2.13^{a}	32.62±1.30^{a}	32.00±1.37^{a}	35.45±7.13^{a}	32.56±2.31^{a}
ΣPUFA	28.46±1.57^{cd}	25.87±3.06^{d}	29.08±1.33^{cd}	34.44±1.24^{b}	34.35±2.48^{b}	42.93±0.77^{a}	31.47±1.23^{bc}	31.39±1.32^{bc}
Σn-3PUFA	17.14±1.45^{c}	16.09±2.14^{c}	17.23±1.13^{c}	21.16±0.83^{b}	21.31±2.63^{b}	28.74±0.81^{a}	21.20±1.09^{b}	18.71±0.90^{bc}
Σn-6PUFA	9.51±0.46^{cde}	8.25±1.21^{c}	10.10±0.38^{bcd}	11.38±1.12^{ab}	10.87±0.14^{abc}	12.01±0.73^{a}	8.78±0.09^{de}	11.06±0.62^{abc}
Σn-3/Σn-6	1.80±0.14^{c}	1.96±0.18^{c}	1.71±0.08^{c}	1.87±0.21^{c}	1.96±0.26^{bc}	2.40±0.19^{ab}	2.42±0.14^{a}	1.69±0.08^{c}

The sea cucumbers were collected from a farm in Qingdao along the coast of the Yellow Sea (China). The different letters show significant difference among the sampling dates ($P < 0.05$). Values are presented as mean ± SD (in % of dry weight.)

From Gao et al., 2011

Table 13.6 Major Fatty Acids in Different Tissues of Sea Cucumber *A. japonicus*

Sampling Sites	Sampling Time	Tissues	Major Fatty Acids (%)	Source
Mori Coast (Southern Hokkaido, Japan)	Oct. 1985	Body wall	16:0 (10.83), 16:1n-7(12.44), 18:0(6.73), 18:1n-7(5.07), 20:4n-6(13.31), 20:5n-3(8.38)	Kaneniwa et al., 1986
Peter the Great Bay, Sea of Japan	Aug. 1985	Body wall	16:0 (11.6), 16:1(16.5), 18:0 (6.4), 20:4n-6 (8.0), 20:5n-3(15.4)	Svetashev et al., 1991
Shikabe (Southern Hokkaido, Japan)	May 1979	Body wall	16:0 (8.20), 16:1n-7(16.96), 18:0(15.98), 18:1n-7(9.26), 20:1n-9(5.92)	Kaneniwa et al., 1986
Aquaculture farm (Dalian Bangchuidao Seafood Enterprise Group Co., Ltd.)	May 2009	Body wall	16:0 (11.34 ± 0.75), 16:1n-7(9.88 ± 0.46), 16:3n3(7.37 ± 0.68), 18:0(8.85 ± 0.45), 20:4n-6(9.62 ± 0.78), 20:5n-3(15.14 ± 0.89)	Sun et al., 2010
		Digestive tract	16:0 (7.51 ± 0.65), 16:1n-7(16.00 ± 0.62), 16:3n3(5.81 ± 0.39), 18:0(7.91 ± 0.57), 18:1n-6(8.30 ± 0.45), 20:5n-3(16.06 ± 0.72)	
Sea of Okhotsk (coast of Abashiri, Japan)	Oct. 2001	Body wall (male)	iso_{15}(6.27), 16:0(8.48), 16:1n-7(6.12), 18:0(6.67), 18:1n-7(6.83), 20:4n-6(10.79), 20:5n-3(7.46), 23:1n-9(5.52), 22:6n-3(5.18)	Kasai, 2003
		Intestine (male)	18:1n-7(7.18), 20:1n9(5.41), 20:4n-6(14.91), 20:5n-3(12.55), 23:1n-9(5.34), 22:6n-3(7.70)	
		Gonad (male)	iso_{15}(5.78), 16:0(6.65), 16:1n-7(5.05), 18:0(5.98), 18:1n-7(7.97), 20:4n-6(8.91), 20:5n-3(9.82)	
		Body wall (female)	iso_{15}(5.85), 16:0(7.35), 16:1n-7(5.19), 18:0(7.58), 18:1n-7(6.98), 20:4n-6(11.01), 20:5n-3(8.01), 23:1n-9(6.36), 22:6n-3(5.14)	
		Intestine (female)	18:1n-7(7.34), 20:1n9(5.34), 20:4n-6(15.20), 20:5n-3(12.47), 23:1n-9(5.69), 22:6n-3(6.25)	
		Gonad (female)	iso_{15}(5.56), 16:0(6.57), 16:1n-7(4.95), 18:0(6.33), 18:1n-7(7.85), 20: 4n-6(8.68), 20:5n-3(9.36)	

Values are presented as mean ± SD (in % of dry weight).

PUFA in *A. japonicus* is consistent with that reported in the abalones *Haliotis laevigata* and *Ha. rubra*, and the scallop *Chlamys farreri* (Su et al., 2006; Xu, 2007).

Gao et al. (2011) showed that the fatty acid 16:0 is the primary SFA, with concentrations varying from 8% (January) to 16% (August) in the body wall of *A. japonicus*; whereas 16:1n-7 is the most abundant MUFA, ranging from 11 to 17% throughout the year. Similar results were also found in some scallops and fishes (Shirai et al., 2002; Wang, 2007).

Monounsaturated fatty acids have received attention in recent years due to their demonstrated beneficial effects relative to cardiovascular heart disease (Kalogeropoulus et al., 2004). The total MUFA levels in the body wall tissues of *A. japonicus* were comparatively high throughout the year, with proportions being higher than PUFA and SFA from March to September and higher than SFA in October, November, and December. Therefore, the relatively stable and high levels of MUFA in the body wall tissues of *A. japonicus* would represent a good source of healthy food supplement for humans.

Eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (AA, 20:4n-6), and docosahexaenoic acid (DHA, 22:6n-3) were identified as the most abundant PUFA in the body wall tissues of *A. japonicus* (Gao et al., 2011). Li et al. (2006) also found high concentrations of AA in *A. japonicus*. In some countries of East Asia, the sea cucumber is a traditional remedy for healing various internal and external wounds (see Chapter 19). AA may play an important role in wound healing as it is a precursor of thromboxane, which influences blood clot formation and attachment to the endothelial tissue during wound healing (Bowman and Rand, 1980).

The proportions of n-3 PUFAs in the body wall of *A. japonicus* (16–29%) were shown to be higher than those of n-6 PUFA (8–12%) (Gao et al., 2011). Furthermore, the ratio of n-3 PUFAs/n-6 PUFAs, which the WHO (World Health Organization) recommends should be no lower than 0.1 in the diet as a whole (Sánchez-Machado et al., 2004), ranged from 1.7 to 2.4 in different seasons. Consequently, the consumption of body wall of *A. japonicus* may be of use to increase this ratio. The ratio of n-3 PUFAs/n-6 PUFAs is between 0.5 and 3.8 in freshwater fishes, and 4.7–14.4 in marine fishes (Henderson and Tocher, 1987; Guler et al., 2008). The ratios in scallops *C. farreri* and *Argopectens irradians* are 1.62–3.94 and 3.45–5.08, respectively (Wang, 2007). An increase in the human dietary n-3/n-6 fatty acid ratio is essential to help prevent coronary heart disease, shock syndrome, cardiomyopathy, and to reduce the risk of cancer (Kinsella et al., 1990).

In conclusion, it seems that geographic location and seasons are important factors affecting the fatty acid composition of *A. japonicus*. Previous studies showed that the diet, degree of maturation, and water temperature are included in the factors that affect fatty acid composition in marine animals (Deng et al., 1976; Morris and Culkin, 1989; Kasai, 2003).

13.4 VITAMINS

Fat-soluble vitamins, including α-VitE, δ-VitE, $VitK_1$, $VitD_2$, and $VitD_3$, and water-soluble vitamins, including $VitB_1$, $VitB_2$, and $VitB_5$, are present in the dry body wall tissues, digestive tract, and female gonad of *A. japonicus* (Table 13.7; Sun et al., 2010). δ-VitE content was clearly variable among different body parts, and the content reached $6229.79 \pm 27.64\,mg\,kg^{-1}$ in the digestive tract (Table 13.7; Sun et al., 2010). α-VitE content in the body wall was higher than in the digestive tract and female gonad. The $VitB_5$ content was different among tissues, with the highest value recorded in the female gonad (Table 13.7; Sun et al., 2010). There was no difference between body wall, digestive tract, and female gonad for contents of $VitK_1$, $VitD_2$, $VitD_3$, $VitB_1$, and $VitB_2$ (Table 13.7; Sun et al., 2010).

Table 13.7 Vitamin Composition in Body Wall, Digestive Tract, and Female Gonad of Fresh *A. japonicus* ($mg\,kg^{-1}$ Dry Weight)

Vitamins	Body Wall	Digestive Tract	Female Gonad
α-VitE	2.67 ± 0.34^a	0.52 ± 0.09^b	0.65 ± 0.08^b
δ-VitE	3411.06 ± 16.59^b	6229.79 ± 27.64^a	475.45 ± 6.13^c
VitK1	1.12 ± 0.20	1.08 ± 0.17	1.16 ± 0.15
VitD2	0.86 ± 0.07	0.71 ± 0.13	0.94 ± 0.17
VitD3	0.73 ± 0.09	0.78 ± 0.15	0.81 ± 0.11
VitB5	2.70 ± 0.36^c	40.40 ± 5.15^b	71.97 ± 4.28^a
VitB1	2.61 ± 0.41	2.75 ± 0.45	2.67 ± 0.50
VitB2	0.69 ± 0.05	1.20 ± 0.22	0.96 ± 0.18

The sea cucumbers were collected in a farm in Dalian (Bangchuidao Seafood Enterprise Group Co., Ltd., China). The different letters show significant differences among the various tissues ($P < 0.05$). Values are expressed as mean ± SD.
From Sun et al., 2010

13.5 MINERALS

In the dry body wall tissues of *A. japonicus* (processed into beche-de-mer), 11 minerals (K, Ca, Mg, Cu, Pb, Cd, Zn, Fe, Co, Ni, and Mn) were found (Li et al., 1989). The contents of Cu, Fe, Mn, and Zn in the dry body wall, digestive tract, and female gonad tissues were all high but differed among the tissues (Table 13.8; Sun et al., 2010). The contents of Cu, Fe, and Mn in the digestive tract was clearly higher than those in the body wall and female gonad. The female gonad was rich in Zn with values reaching $137.44 \pm 1.02\,mg\,kg^{-1}$ (Table 13.8; Sun et al., 2010). The contents of Mg and Ca in the body wall were higher than those in the digestive tract and female gonad (Table 13.8; Sun et al., 2010).

Table 13.8 Mineral Composition of Body Wall, Digestive Tract, and Female Gonad of Fresh *A. japonicus* ($mg\,kg^{-1}$ Dry Weight)

Elements	Body Wall	Digestive Tract	Female Gonad
Cu	0.62 ± 0.04^c	8.46 ± 0.08^a	3.88 ± 0.13^b
Fe	130.99 ± 1.37^b	638.10 ± 5.46^a	101.14 ± 0.36^c
Mn	6.55 ± 0.62^c	50.83 ± 2.23^a	12.23 ± 0.41^b
Zn	38.06 ± 2.23^c	99.03 ± 3.25^b	137.44 ± 1.02^a
Mg	9503.68 ± 3.76^a	2929.98 ± 9.09^b	2569.16 ± 4.66^c
Ca	11792.76 ± 7.06^a	1243.60 ± 11.83^b	1223.62 ± 7.14^b

The sea cucumbers were collected from a farm in Dalian (Bangchuidao Seafood Enterprise Group Co., Ltd., China). The different letters show significant differences among the various tissues ($P < 0.05$). The results are expressed as mean ± SD.
From Sun et al., 2010

There are many other active ingredients in the sea cucumber *A. japonicus*, including polysaccharides, active lipid, and active collagen. Detailed information about these active compounds is provided in Chapter 19.

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CHAPTER

LARVAL, JUVENILE, AND ADULT PREDATORS

14

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SUMMARY

The survival of the sea cucumber *Apostichopus japonicus* in various land-based and field aquaculture systems is negatively correlated with the abundance of potential predators. Different predators affect the various life stages. Copepods, such as *Microsetella* sp. and *Tigriopus japonicus*, can compete for food and space with sea cucumber larvae, and they can also be predators of juveniles <5 mm in body length, thereby causing important mortalities. Moreover, filter-feeders and deposit-feeders (including adults of *A. japonicus* themselves) are potential predators of larvae and newly settled juveniles. Juvenile sea cucumbers with a body length <5 cm can also be preyed upon by crabs, fishes, gastropods, and sea stars, whereas animals with a body length >10 cm have few known predators. Various methods have been developed in an effort to protect the various life stages of *A. japonicus* against predators. In hatcheries and nurseries, trichlorphon can eliminate copepods without harming early life stages. In grow-on ponds and coastal areas, rocks and artificial reefs are used to provide shelter from predators. Bottom trawling, trapping, and manual removal by divers may also be used to decrease the abundance of predators in aquaculture systems.

Keywords: *Apostichopus japonicus*; artificial reef; breeding; copepods; life stage; predator; restocking; sea cucumber; sea star

14.1 PREDATORS OF *Apostichopus japonicus* AT DIFFERENT LIFE STAGES

14.1.1 LARVAL STAGE

The pelagic phase of planktotrophic sea cucumbers, like *A. japonicus*, extends from the fertilized oocyte (egg) to postembryonic stages, which include auricularia, doliolaria, and pentactula larvae (see Chapter 8 for details). The larval stages swim freely in the water column until settlement. In *A. japonicus*, the pelagic phase lasts about two weeks; because the larva moves slowly in the water column, it is easily preyed upon by pelagic predators (some of them being very efficient swimmers). Copepods, such as *Microsetella* sp. and *T. japonicus*, are the main predators during this early life stage (Figure 14.1);

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http://dx.doi.org/10.1016/B978-0-12-799953-1.00014-3

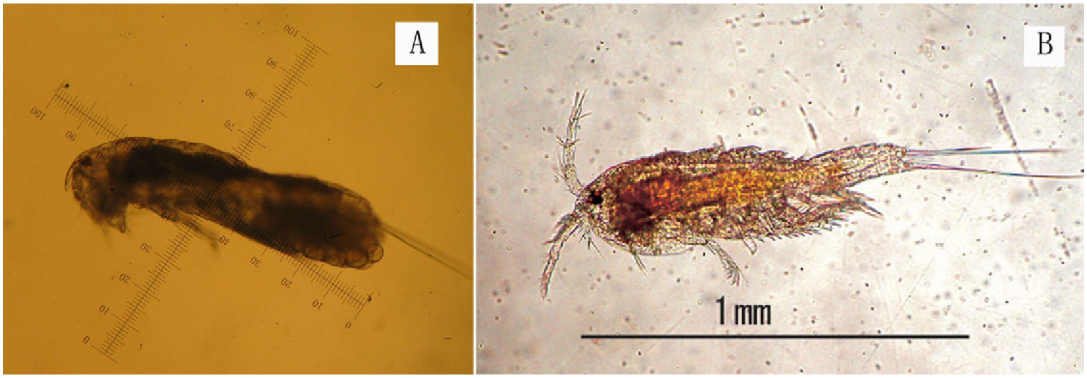

FIGURE 14.1

Predators of *A. japonicus* larvae. (A) The copepod *Microsetella* sp. and (B) the copepod *T. japonicus*.

Photo A from Shilin Liu, Photo B from Noguchi and Noda, 2011

they can injure and kill sea cucumber larvae by bruising their body, and they can also harm the larvae by competing for food and space. The auricularia in *A. japonicus*, which occupies most of the larval stage, is the most vulnerable to predators, similar to the tropical species *Holothuria scabra* (James et al., 1994; James, 2004).

According to the literature, copepods are the most common causative agents of mortalities during the larval period of *A. japonicus* (Liao, 1997; Liu et al., 2004; Li, 2011). The copepods can cause serious harm between May and September, when water temperature is commonly higher than 20 °C. Copepods are more active and reproduce rapidly during this period, which is also coincident with the breeding season of *A. japonicus* (mainly between May and August; see Chapter 6 for gametogenesis and spawning in *A. japonicus*). Algal extracts used to feed sea cucumber larvae can be easily found in the gut of copepods, which suggests competition for food (James et al., 1994; Liu et al., 2002). *A. japonicus* larvae in the presence of a large quantity of copepods assume a ball shape and, in extreme cases, higher mortality rates could be recorded. Because the presence of copepods correlates well with mortalities of sea cucumber larvae during the rearing period, it is advisable to eliminate them from the hatchery cultures as well as from the broodstock tanks where adult sea cucumbers spawn (Kobayashi and Ishida, 1984; Huo et al., 2005).

It was also found that the pelagic gametes and/or larvae of benthic invertebrates can be heavily preyed upon by suspension (filter)-feeding animals, e.g., mussels, scallops, and oysters (Mileikovsky, 1974); therefore, we can envisage that some mortality during the pelagic phase of planktotrophic sea cucumbers in the natural environment (including *A. japonicus*) may be due to predation by filter-feeders. This was demonstrated in embryos and larvae of temperate sea cucumber species (Mercier et al., 2013).

14.1.2 JUVENILE STAGE

14.1.2.1 Main predators of newly settled juveniles

At the end of the larval stage, the pentactula will settle onto a substratum as it completes metamorphosis into the juvenile stage. Again, the most important predators of very early juveniles of *A. japonicus*

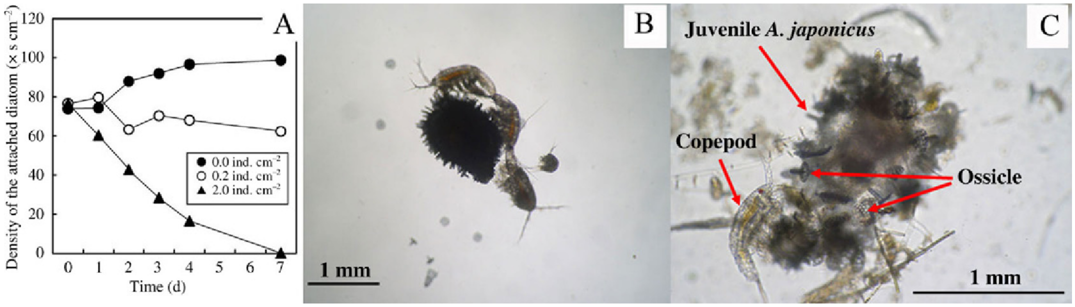

FIGURE 14.2

Harmful effect of the copepod *T. japonicus* on juvenile *A. japonicus*. (A) Temporal variation of diatom density under different levels of abundance of copepods. (B) Copepods feeding on juvenile *A. japonicus*. (C) Disintegrated body of juvenile *A. japonicus* due to copepod predation.

Graph from Noguchi and Noda, 2011. Photos from Saga Prefectural Genkai Fisheries Research and Development Center

are copepods, i.e., *Microsetella* sp. and *T. japonicus*. Among those, *Microsetella* sp. is considered to be of main concern. Unfortunately, the conditions for rearing juvenile sea cucumbers, like those for rearing larvae, can favor the growth and reproduction of copepods. It was found that one adult copepod can produce 90 offspring within 20 days during the breeding season of *A. japonicus* (when water temperature is between 15 and 25 °C). Concurrently, the abundance of sea cucumber juveniles decreases acutely within one or two days following copepod blooms (Kobayashi and Ishida, 1984; Liu et al., 2002; Li, 2011; Noguchi and Noda, 2011). Therefore, efficient methods should be used to eliminate (or control) copepods during these very sensitive culture stages (Wang et al., 2004) (shown later). Copepods can have a harmful effect in the following ways: (1) by competing for food and living space; (2) by injuring the body wall of the juvenile; and (3) by preying directly on the juvenile (Figure 14.2). Copepods are especially harmful to juveniles with a body length <5 mm (Liu et al., 2002, 2004; Noguchi and Noda, 2011) (Table 14.1).

Table 14.1 Effect of Copepod (*T. japonicus*) Density on Survival of *A. japonicus* Juveniles of Various Sizes After a Three-day Trial Period

Copepod Density (ind m^{-2})	Body Length of Juvenile *A. japonicus* (mm) 0.4–1.0	1.0–3.0	3.0–5.0	5.0–10.0
0	15	15	15	15
0.2	7	15	15	15
2	0	10	15	15
7	0	0	15	15
10	0	0	15	15

The initial number of juveniles placed in each trial was 15.
From Noguchi and Noda, 2011

It has been found that the adults of *A. japonicus* decrease their feeding activity during their reproduction to avoid ingesting their own juveniles (Tanaka, 1958; Mileikovsky, 1974), and this may be an effective mechanism for protecting their offspring, as it was also observed in the temperate sea cucumber *Cucumaria frondosa* (Hamel and Mercier, 1996). However, we found that some adult *A. japonicus* continued feeding during the reproduction period, and that the density of juveniles decreased steadily if the adult sea cucumbers had not been removed from the hatchery pond (Liu et al., unpublished data). Therefore, we can suppose that large-sized individuals of *A. japonicus* can prey on or ingest their newly settled offspring (accidentally or otherwise).

14.1.2.2 Main predators of larger juveniles

Juveniles of *A. japonicus* with a body length >1 cm can be used for pond culture and restocking of wild stocks (Tanaka, 2000) (see Chapter 16), but predation on them can still be very high. In fact, predation is one of the major hurdles confronting initiatives concerned with restocking and stock enhancement of sea cucumbers. The main known predators of juveniles of *A. japonicus* in the wild are carnivorous fishes, sea stars, sea urchins, crabs, and gastropods (Preston, 1993; Hatanaka et al., 1994; Sui, 1996; Liao, 1997; Liu, 2000; Tanaka, 2000; Kang and Kim, 2004; Liu et al., 2007; Uekusa et al., 2012) (Figure 14.3).

The size of juvenile sea cucumber selected for release in the wild is important because predation risk generally relates closely to prey size (Hatanaka et al., 1994). It has been suggested that predation on juvenile *A. japonicus* is size-dependent, e.g., small juveniles (<5 cm) are more susceptible to predator-induced mortality than larger sea cucumbers (>10 cm) (Gao, 2011). Among numerous predators of *A. japonicus* in the field, sea stars (e.g., *Asterina pectinifera* and *Asterias rollestoni*, Figure 14.3a, b) are the main ones; they can eat juvenile <5 cm, and their abundance often causes excessive mortality of *A. japonicus* in the wild (Hatanaka et al., 1994; Jiang et al., 2008; Uekusa et al., 2012) as well as in cofferdams. During predation, the sea star approaches the sea cucumber, slowly wrapping its arms around it with the use of tube feet, then everts its stomach and digests it (Figure 14.4). Because sea cucumbers move slowly, they are particularly vulnerable to sea star predation (Hatanaka et al., 1994; Jiang et al., 2008). Hatanaka et al. (1994) demonstrated that the sea star *Asterina pectinifera* (average arm length 43.3 mm) could ingest an average of 1.8, 0.5, and 0.1 *A. japonicus* per day, when the average body length of the prey was 15.9, 30.1, and 40.0 mm, respectively, in the laboratory. Larger sea cucumbers with an average body length of 54.6 mm were not preyed upon (Hatanaka et al., 1994). Moreover, Kang and Kim (2004) documented predation by the trumpet shell *Charonia* sp. on *A. japonicus* measuring ~5.6 cm in length.

The main predators of juvenile sea cucumbers at the intermediate culture stage (<30 mm) are copepods, such as *Microsetella* sp. and *T. japonicus* (similar to larvae and newly settled juveniles, shown earlier). Juveniles of *A. japonicus* measuring 20 mm in body length were also shown to be heavily injured by the crab *Portunus trituberculatus* (43–45 mm in carapace width). Thus, an intermediate culture stage in a more protected environment is recommended for the juvenile sea cucumbers prior to their transfer in any field culture (Sui, 1996). However, larger juveniles are more expensive to produce and may have increased deficiencies induced by longer time spent in a hatchery environment (Sui, 1996; Purcell and Simutoga, 2008).

While the release of hatchery-reared *A. japonicus* juveniles to the natural environment may speed up the recovery of depleted stocks, predation on juvenile sea cucumbers by carnivorous fishes, birds, sea stars, crabs, gastropods, and other invertebrates remains a problem. It is in fact the main disadvantage of sea ranching or sea-pen farming compared to land-based farming in ponds (Hatanaka et al., 1994;

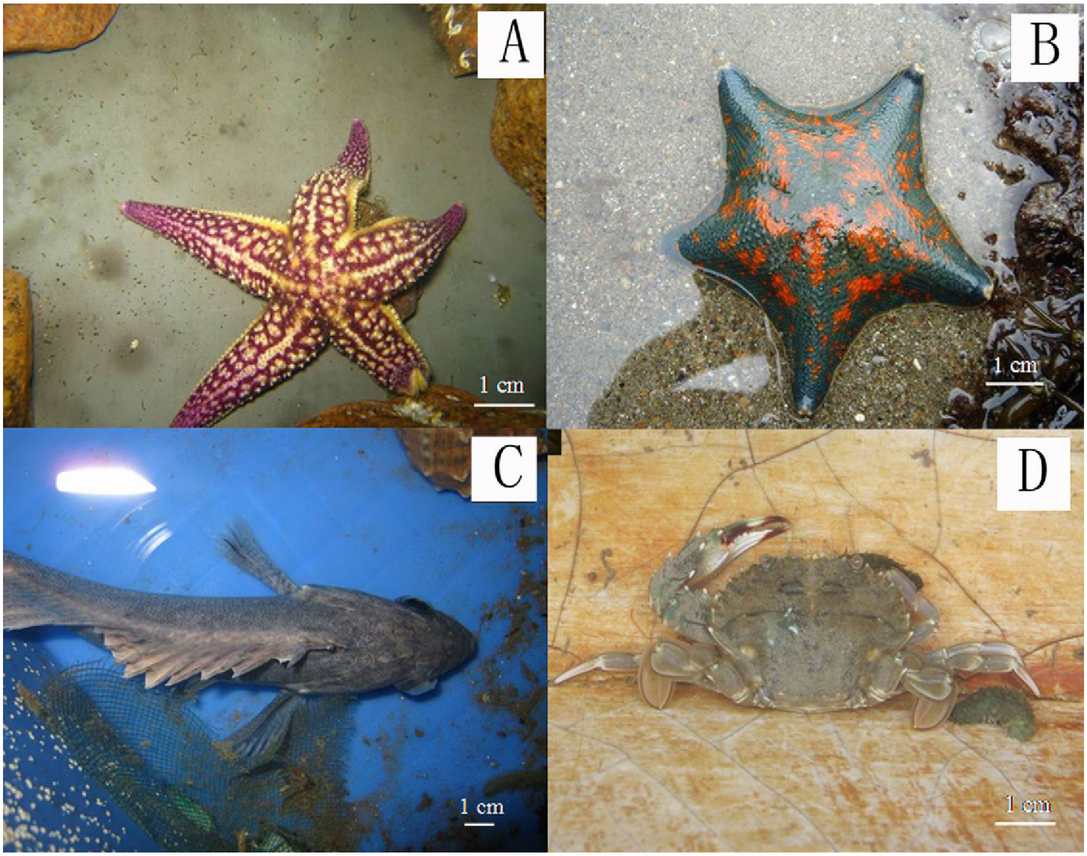

FIGURE 14.3

Main predators of *A. japonicus* <10 cm in length. (A) The sea star *Asterias rollentoni.* (B) The sea star *Asterina pectinifera.* (C) The fish *Sebastodes fuscences.* (D) The crab *Charybdis japonica.*

From Zonghe Yu and Tianlong Qiu

Liao, 1997; Liu, 2000; Jiang et al., 2008; Uekusa et al., 2012), which in turn produce lower quality sea cucumbers with less added value (see Chapter 19 for nutritional and medicinal values) than those grown under natural conditions.

14.1.3 ADULT STAGE

The ability of a prey to avoid predation correlates well with its size. Hence, juveniles are typically more vulnerable to predators than adults (Preston, 1993). Sea cucumbers generally possess one or more antipredation strategies, the main ones being thickness of the body wall (including presence of ossicles), toxic and noxious skin and organs, body swelling or stiffening, evisceration or autotomy (including release of Cuvierian tubules), swimming, strong muscular contractions, nocturnal activity, cryptic or burrowing behavior, and brooding of early life stages (Bakus, 1968; Francour, 1997; Hamel

FIGURE 14.4

The sea star *Asterina pectinifera* preying upon juvenile *A. japonicus.*

From Tianlong Qiu

and Mercier, 2000; So et al., 2010). The sea cucumber *A. japonicus* possesses mutable connective tissue in the dermis, which allows it to alter its rigidity quickly by increasing the stiffness of the body wall, protecting it against predators by enabling it to find support (Tatsuo, 1984). The fact that *A. japonicus* can eviscerate when stressed may be an antipredation measure; however, studies in support of this hypothesis are lacking. Overall, no predators of *A. japonicus* have been confirmed for adult individuals (>10 cm) in the wild (Gao, 2011). Anecdotal reports indicated that seagulls could prey on *A. japonicus* in the intertidal zone and remains of sea cucumbers have also been found in the stomach of salmon and trout (Liao, 1997; Liu, 2000). Moreover, since the trumpet shell *Charonia* sp. was found to prey upon *A. japonicus* measuring ~5.6 cm in length, it might also feed on adult specimens; however, adult sea cucumbers were not tested (Kang and Kim, 2004).

14.2 CONTROL OF PREDATORS IN REARING SYSTEMS

14.2.1 LARVAL AND JUVENILE STAGES

As described previously, copepods are the primary predators of *A. japonicus* during the larval and early juvenile stages. Copepods are particularly dangerous for life stages <5 mm in body length but they can affect juveniles up to 30 mm long. Strategies to eradicate or mitigate the negative impact of copepods are given next.

14.2.1.1 Prevention

Sea cucumber larvae and copepods are similar in size; therefore, it is hard to eliminate them using sieving. However, maintaining high water quality is an effective way to reduce the harm caused by copepods. It has been suggested that an ideal hatchery should be built on the coast where the seawater is clear and clean. Seawater must also be filtered through a gravel bottom filter before being used for

sea cucumber rearing and for microalgal cultures (see also Chapters 7 and 16). In recent years, deep wells located on the shore have been widely used in China to supply clean seawater for rearing of *A. japonicus* (Wang et al., 2004). These water supplies are naturally free of copepods.

The location of the pump for the hatchery should extend offshore to have access to deeper seawater, whereas pumping of near-shore water should be avoided. Compared with natural unfiltered seawater, sand-filtered seawater has low bacterial density, relatively constant pH and fewer copepod counts. The top sand layer of the filter tank must be more than 60 cm thick and seawater should be refiltered through a mesh (75 μm) before its use in the rearing tanks (Liu et al., 2002; Huo et al., 2005).

Apart from water treatment, settlement plates should be cleaned and disinfected before their use. Broodstock food, such as fresh sea mud and grounded and filtered *Sargassum thunbergii*, should be treated with a pesticide (trichlorphon). During the breeding stage, seawater should be changed completely three times a day during summer; the bottom should be cleaned every week to remove feces and uneaten food from the system, and regular transfers of juveniles to clean tanks has also been identified as an effective method to prevent copepod infestations (Liu et al., 2002, 2004) (see Chapter 16).

14.2.1.2 Treatment

Small numbers of copepods are not necessarily harmful during larval and juvenile rearing periods. It was found that juveniles of *A. japonicus* (1 mm in body length) can only be affected by the copepod *T. japonicus* after the diatoms provided as larval food have been completely consumed (Noguchi and Noda, 2011); therefore, addition of algae is an effective means of minimizing predation on the larvae themselves (Huo et al., 2005). However, massive copepod blooms must be treated promptly because, irrespective of the quantity of algae present in the water, they can cause high sea cucumber mortalities. Trichlorphon is the best currently known option to remove copepods (Figure 14.5). A concentration between 2 and 3 ppm is safe for the sea cucumbers but harmful for the copepods; the solution of trichlorphon should be evenly sprinkled into the tanks. All copepods will be killed within 2 h, and the water must be changed completely immediately after the treatment, otherwise prolonged exposure to this chemical could be deleterious for the sea cucumber larvae. Trichlorphon can also be used to eliminate copepods during culture of medium-sized juveniles (James et al., 1994; Yanagisawa, 1996, 1998; Liu et al., 2004; Wang et al., 2004; Gao, 2011; Li, 2011). Long periods of exposure to trichlorphon must be avoided, not only because they can be detrimental for the sea cucumbers but also to avoid the development of resistance by the copepods themselves. Resistant copepods can survive exposure to trichlorphon concentrations of 10 ppm (Huo et al., 2005), which can affect the health of sea cucumbers (of all sizes). As a note, the eggs of copepods cannot be thoroughly killed using trichlorphon (Yanagisawa, 1998), making a total irradiation improbable.

Besides trichlorphon, potassium chloride (KCl, 0.5–1.0% w/w) in association with water jets and selective mesh screens can also be used for copepod control in cultures of juvenile *A. japonicus*. The juveniles are anaesthetized by the KCl solution and become detached from the substrate via a gentle spraying of seawater; they are then easily separated from the copepods by sieving. The use of KCl does not harm juvenile sea cucumbers, but does effectively kill some copepods during the treatment. This method was widely used in Japan for cultivating *A. japonicus* (Yanagisawa, 1996, 1998).

Juveniles affected by copepods often present lesions on the body wall; the cuticle and the skin can be destroyed as well as the upper part of the connective tissue. If visibly affected juveniles are not treated, mass mortality can occur. As soon as injured juveniles become visible in the breeding tank, antibiotics, such as terramycin, acheomycin, sulfamethoxazole, or nitrofurazone, in the range of 3–5 ppm should be used in the pond or other culture vessels every day, which will control and cure the infection

FIGURE 14.5

The pesticide trichlorphon is widely used in China to control copepods in larval and juvenile cultures of *A. japonicus*. (A) Dibaichong™ and (B) Shazaoqing™.

From Zonghe Yu

in two to three days. After the treatment, severe controls should be carried out to avoid the introduction of new copepods to the culture (Liu et al., 2002; Wang et al., 2004; Li, 2011).

14.2.2 GROW-OUT STAGE

Releasing cultured sea cucumbers in the natural environment is an economical and effective way to grow sea cucumbers. However, it was found in *A. japonicus* that the release of small juveniles directly into the wild typically yielded relatively poor results (Sui, 1996; Uekusa et al., 2012). The main reason is that newly released juveniles without intermediate growth in controlled infrastructures are more susceptible to predation, chiefly because of their small size. In order to increase the success of release, a period of intermediate field culture in cages or sea pens is necessary for the seedlings to grow to a larger (safer) size in a more protected environment before entering the ocean. For example, the survival rate of cage-cultured juveniles of *A. japonicus* was very high (80–90%), probably due to the physical protection afforded to the sea cucumbers against predators by the cage itself (Yokoyama et al., 2013).

After this intermediate period of culture in which juveniles reach a larger size, the sea cucumbers are ready to be released in the field for final grow out. However, before release, especially in large ponds and cofferdams, a thorough inspection needs to be done by technicians to remove as many visible predators as possible (e.g., sea stars, crabs), an operation that should continue until the sea cucumbers reach commercial size and are ready to be collected for processing. Three main methods can be used to eliminate sea stars from release areas, i.e., bottom trawling, cage-trap (Figure 14.6A),

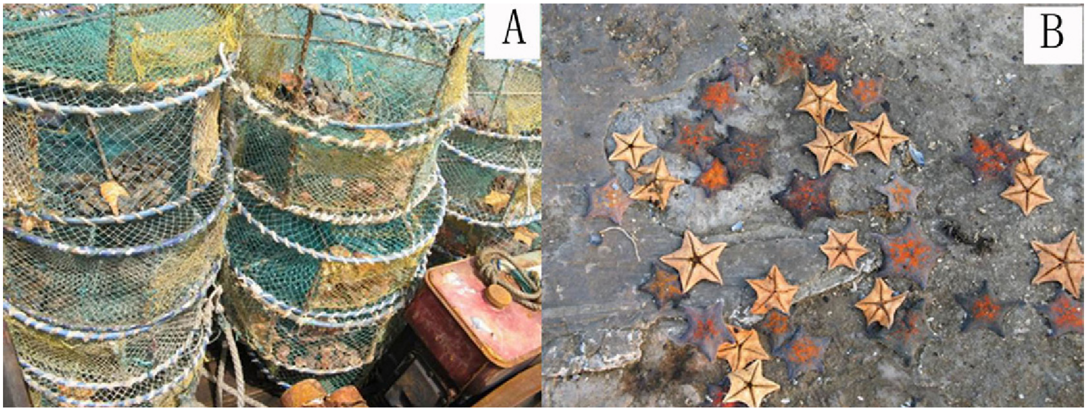

FIGURE 14.6

(A) Cages used to capture predators in culture areas. (B) Sea stars *Asterina pectinifera* are removed and sun dried.

From Kun Xing

or diver collection. Once removed, sea stars are then sun-dried or buried (Figure 14.6B) (Jiang et al., 2008). This monitoring and eradication of potential predators is key in ensuring successful grow out of juveniles (Jiang et al., 2008).

The location of the release site is also instrumental to successful restocking. In the natural environment, *A. japonicus* prefers to live in areas with refuges where rocks or seagrasses abound, whereas few are naturally found on sand or mud bottoms where no refuge exists. In areas with abundant predator populations, an artificial reef system is needed to provide refuge for the sea cucumbers. Artificial reefs can also provide high-quality food for various marine creatures by favoring the growth of benthic microalgae (see Chapter 10). Artificial reefs are usually made from some durable items ranging from stones to tires, concrete rubble, derelict vessels, and other discarded solid materials (see Chapter 18 for the use of artificial reefs). A recently designed "artificial oyster-shell reef" was proven to be effective in restocking sea cucumbers in near-shore areas (Zhang et al., 2011; Zhang and Yang, 2012, see Chapter 18 for details).

14.2.2.1 Farming and sea ranching

Sea cucumber farming and ranching is a key segment of the aquaculture sector. In China, there are three methods used for farming *A. japonicus*: pond culture, pen culture, and sea ranching, sometimes called "bottom culture" (Chen, 2004) (see Chapter 16). In pond culture, tea seed cake (seeds of *Camellia sinensis*) can be used to eliminate predators efficiently by slowly releasing saponin in the water (Figure 14.7) (Liu et al., 2007; Peng, 2009). Artificial reefs made of tiles, bricks, lantern nets, and plastic (Figure 14.8) are also used to protect the farmed sea cucumbers from predators (Chen, 2004). In nearshore areas, net pens, lantern nets, and abalone cages are often used in suspended culture (Figure 14.9). Bottom culture equipment, such as sleeve cages, are also often used (Figure 14.10) to create physical barriers against potential predators (Yu et al., 2014). Released sea cucumbers supplied with shelter from predation were shown to exhibit higher survival rates than sea cucumbers released in areas without any form of protection (Zhang et al., 2011, Zhang and Yang, 2012).

FIGURE 14.7

Tea seed cake used to deter predators in culture ponds.

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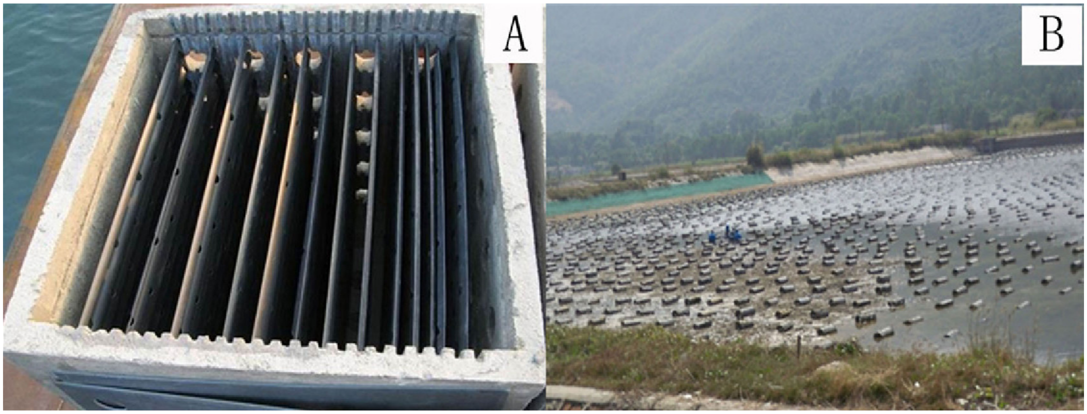

FIGURE 14.8

Artificial reefs provide shelter to *A. japonicus* against predators. (A) The artificial reef system used for restocking and (B) several artificial reef systems deployed in a pond to create a better and safer habitat for sea cucumbers.

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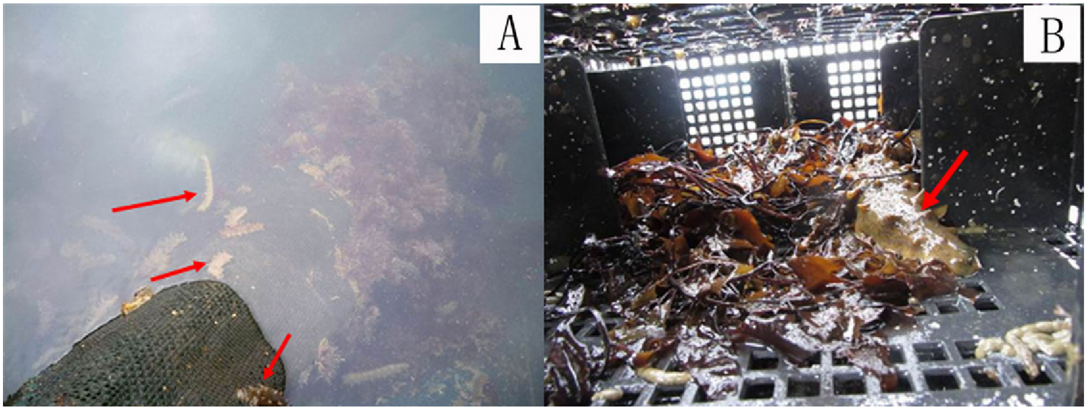

FIGURE 14.9

(A) Net pen and (B) abalone cages used for *A. japonicus* culture. These culture methods are widely used in China to provide shelters against predators. Arrows point to sea cucumbers.

From Zonghe Yu

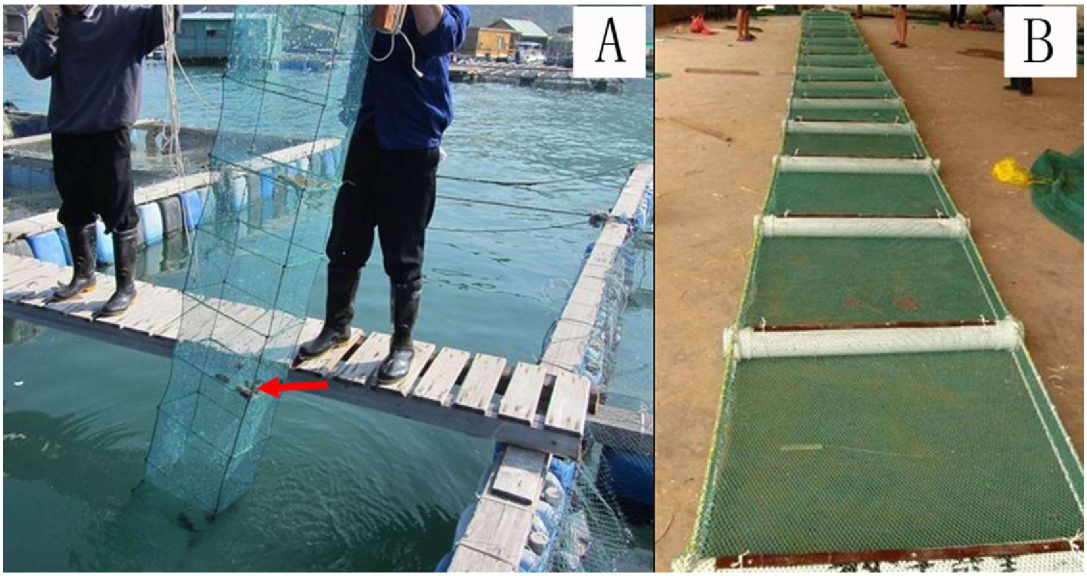

FIGURE 14.10

Bottom culture systems (sleeves) provide shelter against predators for large-sized juveniles of *A. japonicus* during grow out. (A) Sleeves used on soft mud bottoms (arrows point to sea cucumbers) and (B) sleeves used on hard sand bottoms.

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Table 14.2 Main Predators of Different Life Stages of the Sea Cucumber *A. japonicus*, with Suggested Control Method

Life Stage	Predator Species	Impact	Control Methods
Larva	Copepods	Injure and kill larvae; compete for food and space	Maintaining water quality; treating water with trichlorphon
Juvenile (culture)	Copepods	Injure and kill larva; predation; compete for food and space	Maintaining water and food quality; cleaning and disinfecting settlement substrate; treating with trichlorphon; treating with KCl and water jets and selective mesh screens; regular transfer of juveniles to new tanks
Juvenile (released in the field and farming)	Fishes, sea stars, crabs, trumpet shell	Predation	Bring juveniles to a safe size before their release; monitor and remove predators regularly; use of artificial reef; culture with cages, sleeves and other equipment as per antipredator design; treat with tea seed cake to repulse predators
Adult	None confirmed (possibly the trumpet shell *Charonia* sp., salmon, trout and seagull)		

14.3 CONCLUSION

The survival and production of sea cucumbers depends largely on the control of predators at all stages of the culture. Predators and corresponding antipredator methods typical of different life stages of the sea cucumber *A. japonicus* are listed in Table 14.2. Among the major predators, copepods can compete with sea cucumber larvae for food and space and injure small juveniles (<5 mm), sometimes causing mass mortalities. Juveniles with body lengths <5 cm can further be preyed upon by crabs, fishes, and sea stars, whereas adult sea cucumbers >10 cm have few known predators.

The multiplication of copepods can be prevented by pretreatment of seawater and food, and the disinfection of settlement plates. Copepods can be eliminated using trichlorphon, or by isolating the sea cucumbers using a solution of KCl in association with water jets and selective mesh screens. Intermediate culture of seedlings to a refuge size is recommended before release in the field. Predators should be monitored and eliminated both before and after the release of seedlings. Artificial reefs provide refuges and can thereby enhance the success of field release. Tea seed cake can also be used to deter predators. Specialized culture equipment with antipredator design are ideally needed for the culture of sea cucumbers at sea in order to optimize survival rates to the commercial size.

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CHAPTER

IMMUNOLOGY AND DISEASES

15

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SUMMARY

Knowledge of the immune defenses in Holothuroidea, and more generally in Echinodermata, is considered to be important due to their phylogenetic vantage point, which can help infer both the early evolution of bilaterian immunity and the underpinnings of the vertebrate adaptive immune system. It is also crucial to determine how immunological functions are affected by environmental stresses. The first segment of this chapter details cell types in the coelomic and hemal systems of sea cucumbers and considers the influence of environmental stressors on the immune response of *A. japonicus*. The second segment focuses on the response to pathogens. With the rapid expansion and intensification of sea cucumber farming, the incidence of diseases has increased and causative agents have been investigated in various life stages. This chapter discusses symptoms and treatments of various infections, including the syndromes of rotting edges and ulceration of the stomach in auricularia larvae, autolysis of young juveniles, as well as skin ulceration, epidermis erosion, and body edema of adults triggered by bacteria, fungi, and parasites.

Keywords: *Apostichopus japonicus*; coelomocyte; disease; environmental stress; hemocyte; immunological function; treatment

15.1 THE IMMUNE SYSTEM OF SEA CUCUMBERS

Sea cucumbers, including *Apostichopus japonicus*, are members of the class Holothuroidea and phylum Echinodermata. Immunological reactions in sea cucumbers involve cellular and humoral immune responses, which have evolved to protect animals from invading pathogens and to repair damaged tissues. The immunological response is primarily carried out by coelomocytes, which mediate the cellular response to immune challenge through phagocytosis, encapsulation, cytotoxicity, and the production of antimicrobial agents. The humoral immune system includes immune factors in body fluids, such as lysozyme, complementoid, and lectin (Smith et al., 1994; Gross et al., 1999; Zhang, 2005).

15.1.1 TAXONOMY OF COELOMOCYTES

The body cavity in sea cucumbers contains a fluid, i.e., coelomic fluid, in which coelomocytes are suspended. In the absence of specific immune tissues and organs, the cellular and humoral immune

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responses are performed by coelomocytes (Eliseikina and Magarlamov, 2002). However, coelomocyte composition varies widely among different specimens, and the types of coelomocytes may even vary among coelomic compartments within a single sea cucumber (Hetzel, 1963). The diversity and classification of echinoderm coelomocytes has thus been underestimated, and the assignment of cell types to specific roles is currently inadequate. There are therefore no uniform criteria for the classification of coelomocytes, probably because of the animal's varied developmental stages and physiological states.

The cellular composition of the coelomic fluid in the sea cucumber *A. japonicus* has been studied using light and transmission electron microscopy (TEM) and histochemistry, which identified progenitor cells, amebocytes, vacuolated cells, small (or "young") morula cells, small, small I and small II morula cells, crystal cells, and vibratile cells (Eliseikina and Magarlamov, 2002). Large and small granule cells, transparent cells, and lymphoid cells have also been observed in *A. japonicus* by Liu et al. (2005) using transmission electron microscopy. Granule cells are the most abundant, transparent cells are anomalous and less abundant than granule cells, with a large nucleolus and pseudopods on the facies, while lymphoid cells are the least common (Li et al., 2009b). Xing et al. (2008) also characterized six types of coelomocytes by light microscopy including lymphocytes, morula cells, amebocytes, crystal cells, fusiform cells, and vibratile cells (Figure 15.1). Fresh preparations and Wright-stained coelomocyte smears distinguished hyalinocytes, granulocytes, acidophilic, and basophilic cells among the coelomocytes. In Wright-stained smears, two coelomocyte types, hyalinocytes and granulocytes, were identified based on the presence or absence of cytoplasmic granules, respectively. Two types of hyalinocytes (large and small) were also found to differ greatly in terms of their shape and content. The presence of both large and small hyalinocytes suggests that they might either represent two different developmental stages of the same cell type, or two different types of hyalinocytes with similar staining properties.

Small hyalinocytes contained a central nuclei and scanty cytoplasm, with a mean size of 3.0 μm (Table 15.1). Large hyalinocytes varied greatly in diameter, ranging from 3.0 to 9.6 μm, their single, round nucleus was located on one side of the cytoplasm, and they had a mean diameter of 2.9 ± 0.08 μm (± SD; range: 2.4–3.6 μm). Granulocytes were also determined to be either small or large. Differences in cell size were found among the four types of coelomocytes, except in the case of large hyalinocytes and small granulocytes (Table 15.1).

The concentration of coelomocytes in *A. japonicus* was $3.79 \pm 0.65 \times 10^6$ cells ml^{-1} (mean ± SD). Coelomocytes comprised 76.69% hyaline cells and 23.31% granular cells, according to the presence or absence of granules (Table 15.2).

Three subpopulations were distinguished according to partial size, as analyzed by flow cytometry. Group R1 cells were relatively large with low densities and corresponded to large granulocytes (Table 15.2). R2 cells were intermediate in size between R1 and R3, and may represent aggregations of small granulocytes and large hyalinocytes. R3 cells were much smaller and more abundant, corresponding to small hyalinocytes (Xing et al., 2008).

Five types of coelomocytes were identified by scanning electron microscopy (SEM), on the basis of their size and shape (Figure 15.2) (Xing et al., 2008):

1. Round cells: These cells might correspond to lymphocytes identified by light microscopy. They were small (about 2–4 μm) and abundant in all cell types. They were round with a relatively smooth surface (Figure 15.2A), occasionally with two pseudopodia at the opposite poles of the cell (Figure 15.2B). "Triangular" round cells had a filiform pseudopodium extending from each corner (Figure 15.2C).

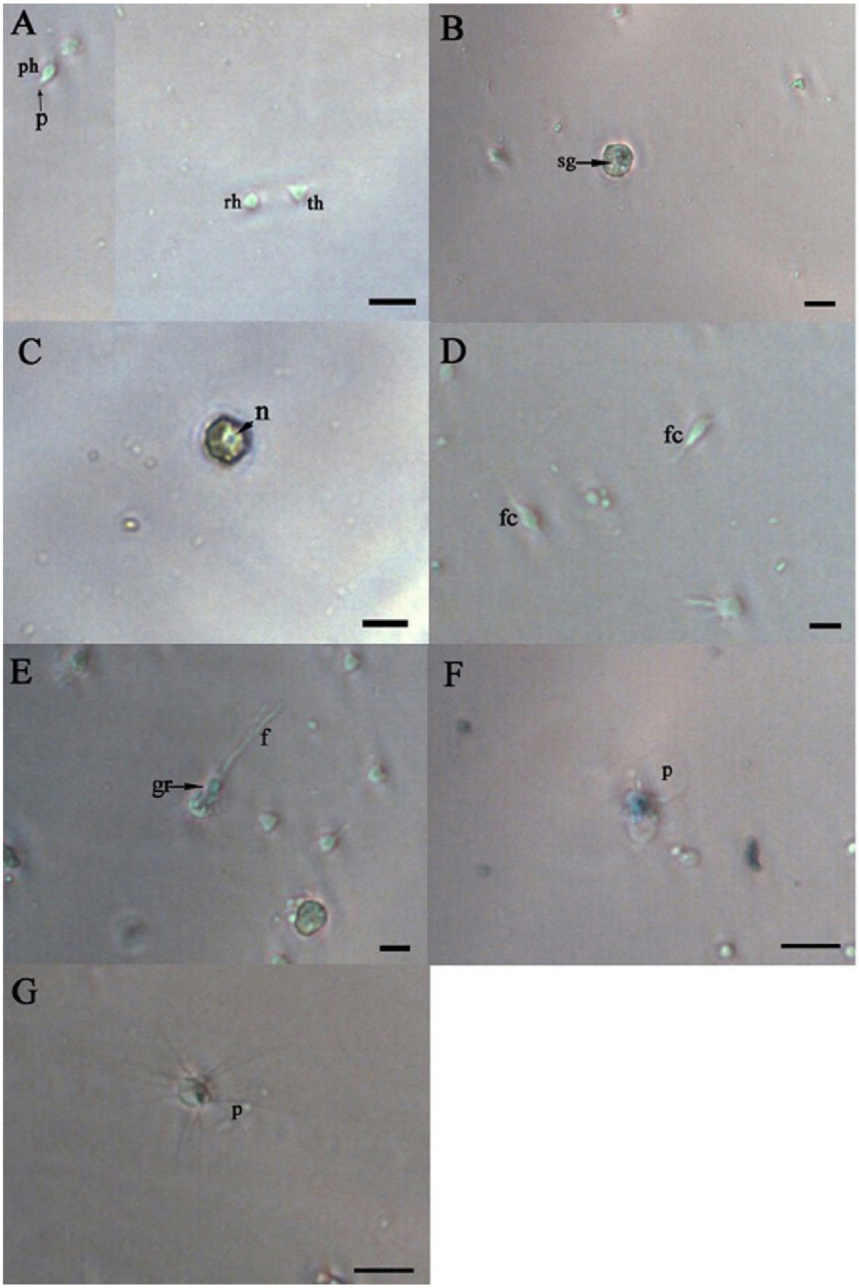

FIGURE 15.1

Light micrographs of *A. japonicus* coelomocytes. (A) Small hyalinocytes (scale bar: 10 μm); (B) large granulocyte (scale bar: 10 μm); (C) crystal cell (scale bar: 5 μm); (D) fusiform cells (fc) (scale bar: 5 μm); (E) vibratile cell (scale bar: 5 μm); (F) petaloid amebocyte (scale bar: 10 μm); (G) filiform amebocyte (scale bar: 10 μm). rh, round hyalinocyte; ph, hyalinocyte with two pseudopodia; th, triangular hyalinocyte; p, pseudopodia; sg, secretory granules; n, nucleus; gr, granule.

From Xing et al., 2008

Table 15.1 Diameter of Four Types of Coelomocytes in the Coelomic Fluid of *A. japonicus*

	Cell Diameter (μm)		
Coelomocytes Type	**Mean ± SE**	**Minimum**	**Maximum**
Small hyalinocytes	3.01 ± 0.09	2.10	3.90
Large hyalinocytes	5.47 ± 0.15	3.00	9.60
Small granulocytes	5.83 ± 0.41	4.50	6.60
Large granulocytes	10.51 ± 0.24	9.00	12.90
Total	6.20 ± 0.29	2.10	12.90

From Xing et al., 2008

Table 15.2 Percentage of Granulocytes and Hyalinocytes Found in the Coelomocyte Population of *A. japonicus*

Type of Cell	Mean	SE	*N*	Sum	%	Total
Small hyalinocytes	61.75	3.24	20	1235	59.83	76.69
Large hyalinocytes	17.15	1.53	20	343	16.85	
Type I granulocytes	12.25	1.29	20	245	11.89	23.31
Type II granulocytes	11.90	1.19	20	238	11.42	

N = *Number of view fields, distinguished and counted on Wright's stained smears.*
From Xing et al., 2008

2. Amebocytes: These cells were about 5 μm in diameter with various cytoplasmic pseudopodia on the surface (Figure 15.2D).
3. Morula cells: These were spherical in shape, ranging in diameter from 8 to 20 μm. The small spherules inside were of uniform size (0.5–2 μm diameter) and completely filling the cells (Figure 15.2E).
4. Crystal cells: These were multifarious in shape, including styloid, cuboid, hexagonal, and other shapes (Figure 15.2F–I). The round nucleus was located at the periphery of the cell.
5. Fusiform cells: These were usually pear- or spindle-shaped, with relatively smooth surfaces, with two long pseudopodia located at opposite ends of the cell (Figure 15.2J). Fusiform cells ranged from 2 to 4 μm in diameter and from 15 to 20 μm in length.
6. Vibratile cells were not observed under scanning electron microscopy.

Four coelomocyte types could be determined by TEM observation (Figure 15.3) (Xing et al., 2008):

1. Lymphocytes: These were spherical cells about 3 μm in diameter. A single, finely granulated spherical nucleus almost filled the entire cell (Figure 15.3A). These cells might correspond to the small hyalinocytes seen with light microscopy and the round cells identified by scanning

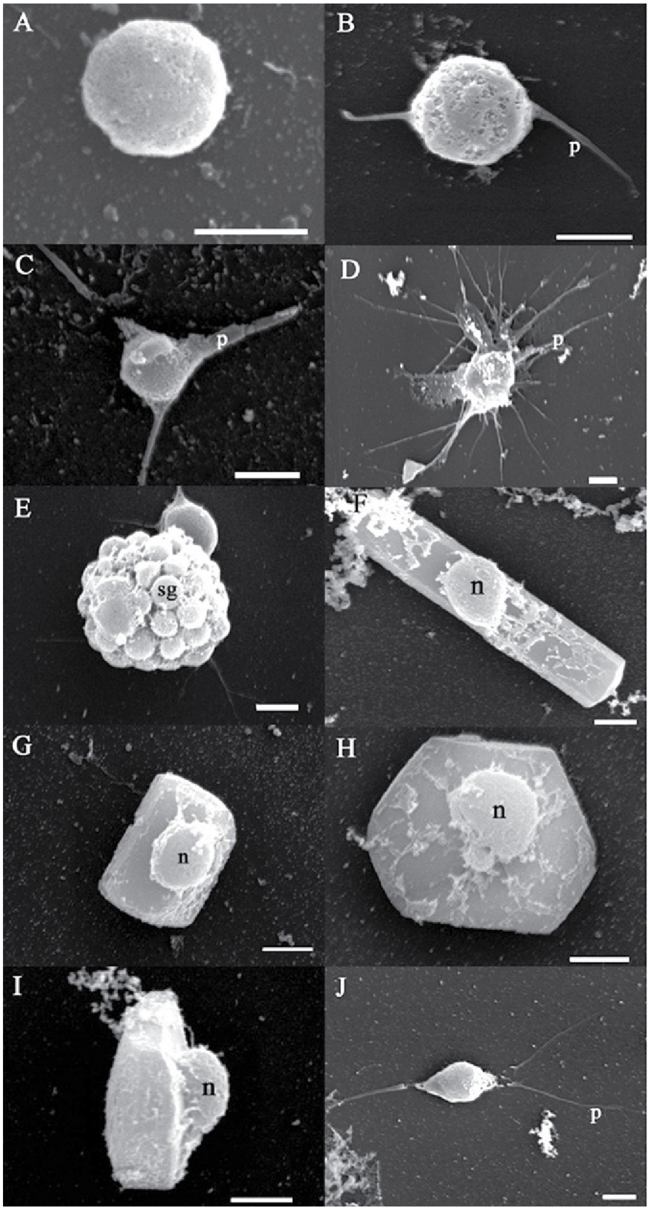

FIGURE 15.2

Scanning electron micrographs of *A. japonicus* coelomocytes. (A) Round cell; (B) round cell with two pseudopodia; (C) round cell with three pseudopodia; (D) amebocyte; (E) morula cell; (F) styloid crystal cell; (G) cuboid crystal cell; (H) hexagon crystal cell; (I) lateral view of a crystal cell; (J) fusiform cell. p, pseudopodia; sg, secretory granule; n, nucleus. Scale bars: 2 μm.

From Xing et al., 2008

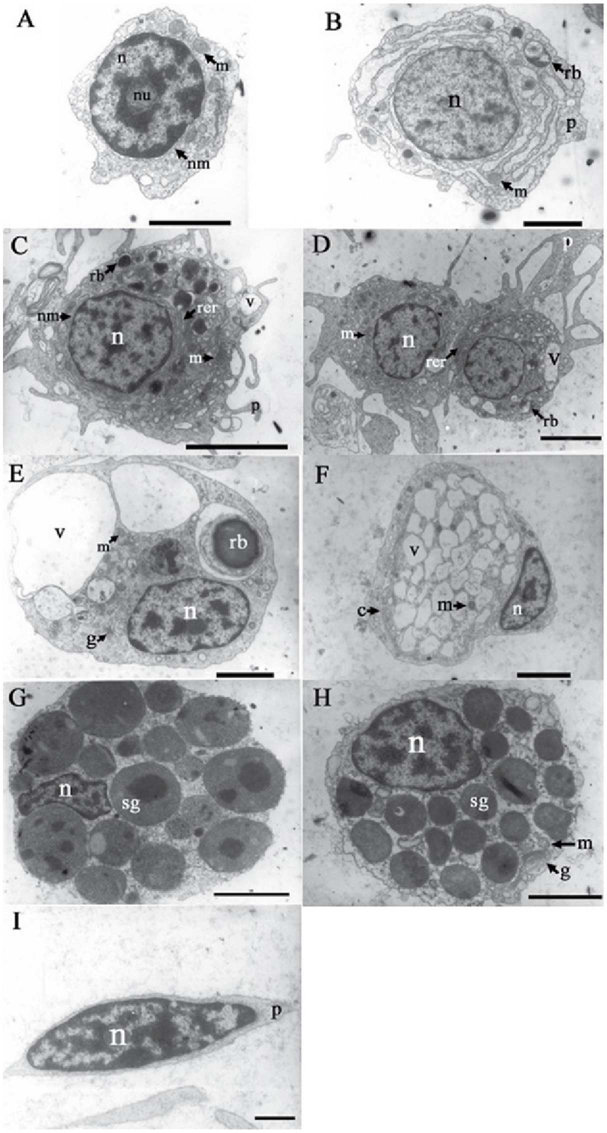

FIGURE 15.3

Transmission electron micrographs of *A. japonicus* coelomocytes. (A) Lymphocyte with thin cytoplasm; (B) petaloid amebocyte; (C) filiform amebocyte; (D) clotting of amebocytes; (E) an old cell phagocytosed by an amebocyte; (F) void morula cell phagocytosed by an amebocyte; (G) heterogeneous granules of a morula cell; (H) homogenous granules of a morula cell; (I) fusiform cell; (J) vibratile cell. n, nucleus; nu, nucleoli; nm, nucleus membrane; m, mitochondria; p, pseudopodia; rb, residual bodies; rer, rough endoplasmic reticulum; v, vacuoles; g, Golgi complex; c, cystozooid; sg, secretory granules. Scale bars: 2 μm.

From Xing et al., 2008

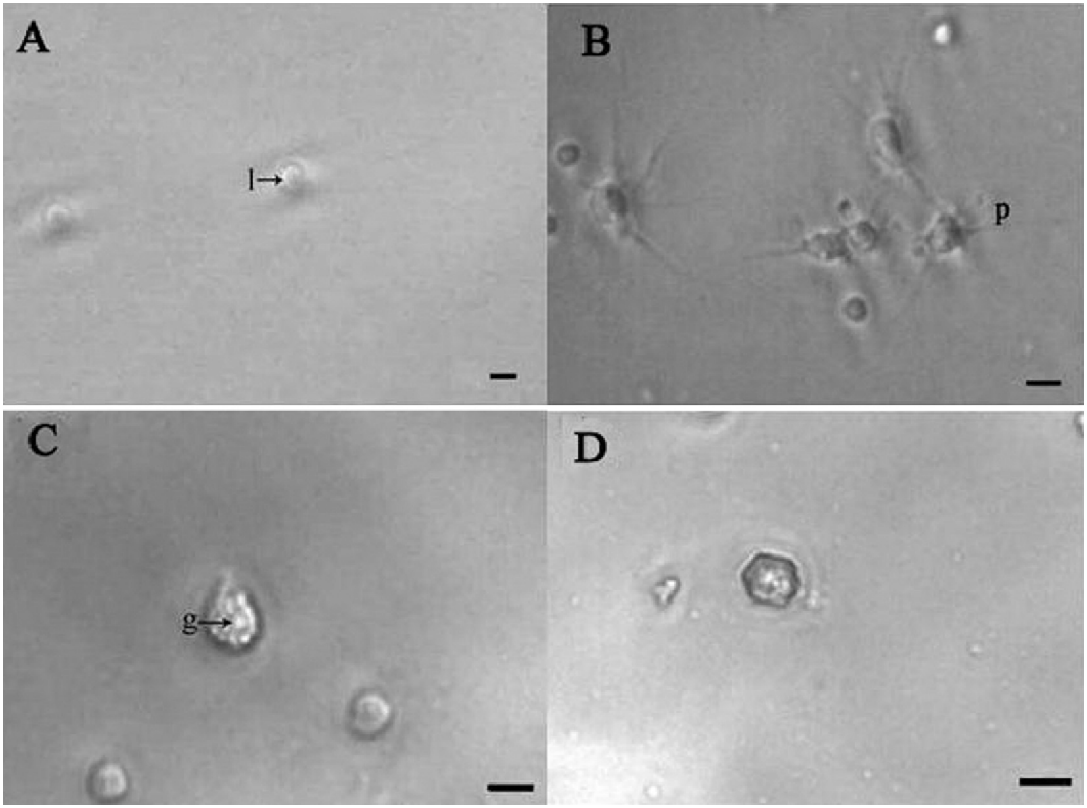

FIGURE 15.4

Light micrographs of coelomocytes from *A. japonicus.* (A) Lymphocyte; (B) amebocyte; (C) morula cell; (D) crystal cell. 1, 1ymphocyte; g, granule; p, pseudopodia. Scale bars = 2 μm.

From Xing and Yang, 2008

electron microscopy. A single electron-dense nucleolus was occasionally visible. The nucleus was surrounded by a thin ring of dense cytoplasm including some mitochondria and several vacuoles.

2. Amebocytes: These cells possessed clear hyaline peripheral cytoplasm that produced pseudopodia, and a granular perinuclear cytoplasm containing the single nucleus and various cytoplasmic inclusions. All amebocytes possessed either petaloid or filiform pseudopodia (Figure 15.3B–C) that radiated in different directions from the central perinuclear cytoplasm of the cells. Amebocytes were capable of clotting with filiform pseudopodia (Figure 15.3D). The perinuclear cytoplasm contained rough endoplasmic reticulum, mitochondria, numerous vacuoles, and phagosomes with electron-dense contents. Residual bodies (Figure 15.3E) were also abundant in the cytoplasm. Amebocytes were thought to phagocytose foreign particles and old cells. An entire degraded cell was shown enveloped by a plasma membrane, which involved the formation of a cystozooid (Figure 15.3F).

3. Morula cells: These were spherical in shape and filled with large secretory granules, mitochondria, and Golgi complexes (Figure 15.3H). They contained little cytoplasm, because most of the cell was occupied by densely packed secretory granules. The nucleus was usually irregular in shape. The granules in these cells differed in content; type I morula cells contained heterogeneous granules with a central electron-dense core and a loose fibrillar shell (Figure 15.3G), while type II morula cells possessed homogenously electron-dense granules (Figure 15.3H).
4. Fusiform cells: These possessed two long pseudopodia tapering from the ends of the cells (Figure 15.3I), which exhibited a regular discoid shape.
5. Crystal cells were not observed under transmission electron microscopy.

Although great variation and confusion existed among those descriptions, within these major categories, several distinctive cell types occurred that might represent different developmental stages and different physiological status.

15.1.2 TAXONOMY OF HEMOCYTES

Various cell types suspended in the body fluid of sea cucumbers have been designated as coelomocytes or hemocytes, depending upon whether the fluid occupied a pseudocoelomic or coelomic space, or was confined within the hemal system. Open or closed cells suspended in the hemal space confined within dorsal and ventral vessels were designated as hemocytes (Mary, 1972). Hemocytes in the hemal vessels resembled coelomocytes in the coelomic fluid, with identical morphology and structure, but their density was lower with less diversity.

Hemal (blood) vessels in the sea cucumber *Stichopus moebi* are reported to consist of a coelomic surface of ciliated epithelium, a layer of muscle and nerve cells, followed by connective tissue and the luminal lining of endothelium (Herreid et al., 1976). For comparative purposes, the hemal system of *A. japonicus* and its function were investigated using light microscopy, and scanning and transmission electron microscopy (Liu et al., 2004). The vasculature was composed of a vascular epithelium, basement membrane, and vascular lumen. Vascular epithelial cells were arranged tightly and the basement membrane was continuous and stratified, creating a continuous and closed vascular net. Microvilli were present on the free surface of the vascular epithelial cells.

The hemal system in the sea cucumber is a well-developed closed circulatory system. It consists of two major vessels running parallel to the gut, of which the dorsal vessel is more prominent. The dorsal vessel pumps colorless fluid via the vessels within the walls of the intestine into the ventral vessel, and hemolymph passing from the dorsal pulmonary vessel can connect to the gut (Liao, 1997) (see Chapter 4). Large and small granule cells, transparent cells, and lymphoid cells were recognized by TEM (Liu et al., 2004), while lymphoid cells, spherulocytes, amoeboid phagocytes, hyaline cells, and fusiform cells were detected by light and electron microscopy (Li et al., 2009). Four different types of cells have been recognized from the dorsal hemal vessel, which can be described by their morphological characteristics using light and scanning electron microscopy: lymphocytes, amebocytes, morula cells, and crystal cells were observed by staining living coelomocytes, tissue sections, and ultrathin sections, using modified Wright's staining (Figures 15.4 and 15.5) (Xing and Yang, 2008). Lymphocytes were spherical, generally smooth in outline, about 2 μm in diameter, and were numerically dominant. Amebocytes possessed various cytoplasmic pseudopodia, were about 5 μm in diameter, and were capable of clotting with other hemal cells. Morula cells were spherical in shape, ranging in diameter from 5 to 20 μm, according to the size of granules.

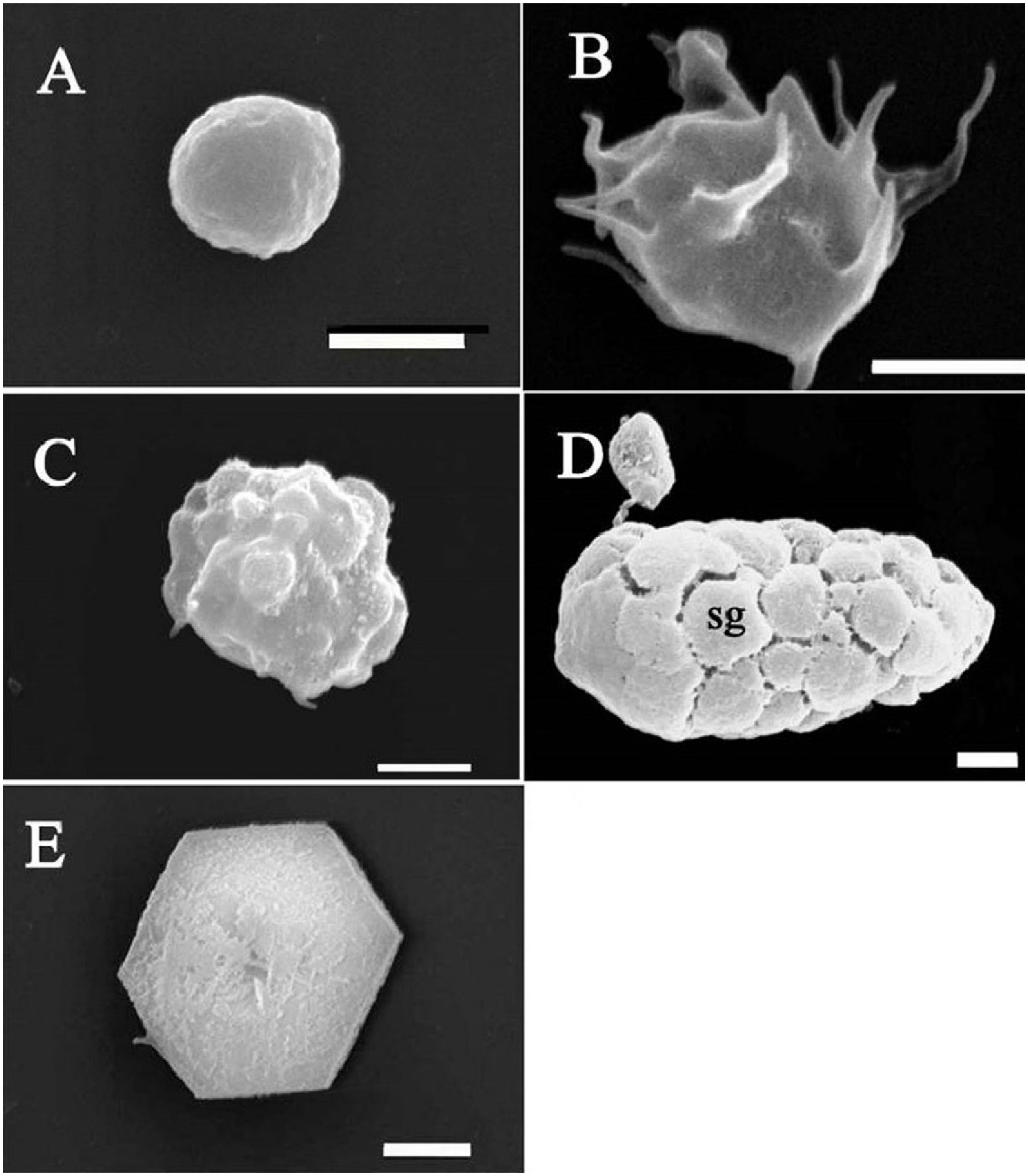

FIGURE 15.5

Scanning electron micrographs of hemocytes from *A. japonicus*. (A) Lymphocyte; (B) amebocyte; (C) small morula cell; (D) 1arge morula cell; (E) crystal cell. sg, granule. Scale bars = 2 μm.

From Xing and Yang, 2008

15.1.3 ORIGIN AND FUNCTION OF COELOMOCYTES

As in other echinoderms, the free coelomocytes in the coelomic cavity of sea cucumbers are thought to participate in gas exchange, nutrient transport and storage, clot formation, excretion of waste products, production of connective tissue components, and immune defense (Mary, 1972; Eliseikina and Magarlamov, 2002). Fusiform and vibratile cells appeared to be either artifacts or cells foreign to the coelomic cavities, and the relative members of coelomocyte types varied among coelomic compartments within a given specimen.

Previous studies and current research suggest that only lymphocytes, amebocytes, morula cells, and crystal cells are common cellular elements in the coelomic fluid of *A. japonicus*, whereas fusiform and vibratile cells are not present in all specimens (Xing et al., 2008). See Section 15.1.1 for details on the taxonomy and various types of coelomocytes.

Lymphocytes were determined to be numerically dominant in stained smears (Xing, 2009). They were relatively smooth, lacked complex pseudopodia, and typically had a prominent nucleus with moderately electron-dense chromatin. This suggests that the lymphocytes were poorly differentiated and may therefore be the precursors of some types of coelomocytes. Accordingly, lymphocytes have often been called progenitor cells (Chia and Xing, 1996; Eliseikina and Magarlamov, 2002).

Amebocytes are the second most abundant cell type in the coelomic fluid in *A. japonicus* and were interpreted to be amoeboid phagocytes (Xing, 2009). Amebocytes contain heterogeneous material of various sizes. They often display many pseudopodia, and amebocytes with numerous pseudopodia often clump together. In *A. japonicus*, as well as in other echinoderms, amebocyte pseudopodia are petaloid and filiform (Edds, 1993). Canicatti et al. (1989) described dynamic transformations from petaloid to filiform stages in *Holothuria polii*. Petaloid-shaped phagocytes were capable of direct transformation into amebocytes with filiform pseudopodia, but the transformation of filiform pseudopodia into petaloid pseudopodia has never been observed (Hetzel, 1963). Careful observation of fresh preparations revealed that all amebocytes possessed pseudopodia that radiated in different directions from the central perinuclear cytoplasm of the cell (Xing, 2009). Amebocytes undergo these morphological changes before and during cell aggregation; in fresh preparations, the petaloid pseudopodia appear to collapse, and a redistribution of the cytoplasm produces branching filiform pseudopodia of considerable length, often intermingling with the filiform pseudopodia of other amebocytes, referred to as clotting. Clotting is thought to be a defense mechanism against loss of coelomic fluid when the body wall is punctured (Mary, 1972). Amebocytes also play a role during the cellular encapsulation of foreign materials and microbes (Hillier and Vacquier, 2003). The residual bodies abundant in the cytoplasm may be old or damaged coelomocytes, and elimination of these cells from the coelomic fluid by amebocytes was a constant process.

Morula cells are a common coelomocyte type in the coelomic fluid with cytoplasmic granules (Xing, 2009). These cells have also been found in ascidians and the dendrochirotid sea cucumber *Eupentacta quinquesemita* (Ballarin et al., 1995; Byrne, 1986; Cima et al., 2006). Morula cells were characterized by a compressed and even irregular nucleus, and numerous electron-dense granules containing proteins, suggesting that they might be secreted and function as extracellular digestive enzymes (Massin, 1980). Byrne (1986) described the ultrastructure of morula cells in the dermis of *E. quinquesemita*, and considered that these cells functioned in the maintenance of the extracellular matrix as a source of ground substance material. Morula cells were referred to as trephocytes, which participate in food storage and the synthesis of many humoral factors in the echinoderm immune system and extracellular matrix of connective tissue. Morula cells were divided into different types: type I cells contained

heterogeneous granules with a central electron-dense core, consisting of proteins, lipids, and neutral polysaccharides, surrounded by a loose fibrillar shell composed of acid polysaccharides (Massin, 1980; Eliseikina and Magarlamov, 2002). Type II cells included homogenously electron-dense granules consisting of glycoproteins (Eliseikina and Magarlamov, 2002). In addition to these two morula cell types, cells with vacuoles similar in size to the granules are also detected, but appeared to lack contents at the ultrastructural level, with no electron-lucent granules stained by any of the vital dyes used. Comparison of the morphology and structure of the three different types of morula cells revealed strong similarities, continuity, and coherence.

Crystal cells exhibit various shapes, including styloid, cuboid, and hexagonal, among others. The thin layer of hyaline cytoplasm surrounding the crystal cells is only visible when viewed from a favorable angle. The single nucleus was often flattened and closely applied to the crystal cells. Hetzel (1963) reported that crystal cells were not detectable in fixed samples because they dissolved under slight osmotic stress, or were lost during the staining and dehydration processes. However, Xing et al. (2008) identified crystal cells using scanning electron microscopy, not transmission electron microscopy. Crystal cells in *A. japonicus* were larger and more complex than in other holothurians, suggesting a role in osmoregulation (Eliseikina and Magarlamov, 2002). Changes in osmotic pressure result in reversible crystallization of the intravacuolar material, thereby normalizing the osmotic pressure. Hetzel (1963) suggested that crystal cells were related to the mesenchymal ossicle-forming cells of the body. However, further studies are needed to confirm these hypotheses.

Apart from the common types of coelomocytes just mentioned, fusiform and vibratile cells have distinctive appearances and limited distributions among species. These cell types are occasionally observed in fresh preparations of coelomic fluid, and their relative numbers increased with repeated handling and sampling from a given specimen (Xing and Yang, 2008). Two long pseudopodia tapering at the ends of the cells were evident in fusiform cells. Vibratile cells in living preparations were similar in structure and size to young amebocytes, but possessed a long flagellum and swam rapidly through the fluid in living preparations. The origin of vibratile cells is unclear; Hetzel (1963) considered that these irregular cells were contaminants of some kind or protozoan symbionts. Surface contamination of *A. japonicus* could have been introduced into the sample by the needle inserted into the organism. Mary (1972) considered that vibratile cells originated in the peritoneum, while Lecal (1980) observed an endoparasitic flagellate of the genus *Cryptobia* in the coelomic fluid of the crinoid *Antedon bifida*. Matranga et al. (2006) described round vibratile cells with a long flagellum moving in a straight direction along a helicoidal path in the sea urchin *Paracentrotus lividus*. However, few researchers have studied the morphology of vibratile cells because of their fast movement (Matranga and Bonaventura, 2002), and their function thus remains unclear. An attractive hypothesis suggests that vibratile cells contribute to the continuous circulation of the perivisceral coelomic fluid generated by the ciliated coelomic epithelium (Ruppert and Barnes, 1994). Vibratile cells had longer and more conspicuous flagellum-like extensions that were markedly different from flagellated endoparasites; the appearance of vibratile cells has also been interpreted as developing amebocytes in the peritoneum (Mary, 1972). These interpretations have established vibratile cells as endogenous members of the coelomocyte population, and it has been suggested that they may function to keep the coelomic fluid in motion by lashing their vibratile processes.

Hemocytes containing hemoglobin are found extensively in the perivisceral coelom and the water vascular system in members of the class Holothuroidea. They were shown to be widely distributed among members of the orders Dendrochirotida and Molpadida, but Aspidochirota, including *A. japonicus*,

were devoid of hemocytes, as previously reported (Hetzel, 1963; Robert and Nora, 1988; Eliseikina and Magarlamov, 2002). Further studies are needed to elucidate the structure and function of the various coelomocyte types in order to further our understanding of the immunology and physiological ecology of *A. japonicus*.

Coelomocytes are produced by a pseudostratified layer of coelomic epithelial cells that covers the interior of the body wall, the outer surfaces of the digestive tract, and the lining epithelium of the respiratory tree. Regarding their origin, several additional factors support the hypothesis that these cells may be derived from the lymphocytes that also give rise to amebocytes. The lymphocytes originate from the lining epithelium of the respiratory trees and migrate in the coelomic fluid, and differentiate into morula cells and possibly other coelomocytes (Endean, 1958). In accord with this theory, the sparse lymphocyte cytoplasm contained few mitochondria that were relatively smooth in outline, with small, unbranched pseudopodia that were less complex than the branching filiform pseudopodia of amebocytes. Fusiform and vibratile cells were able to produce pseudopodia of some forms (Hetzel, 1965; Eliseikina and Magarlamov, 2002). Although Hetzel (1963) considered these irregular cells as contaminants or protozoan symbionts, the appearance of vibratile cells was interpreted as a stage in the development of amebocytes in the peritoneum. Hetzel established them as endogenous and true, albeit scarce, members of the coelomocyte population, which maintained the coelomic fluid in motion by lashing of their vibratile processes. The origin and characterization of vibratile cells requires further verification.

Quantitative analysis of phagocytosis by amebocytes in *A. japonicus* was studied by light and electron microscopy using carmine, yeast, and zymosan A as markers (Chia and Xing, 1996; Xing and Yang, 2009). Coelomocytes demonstrated the ability to agglutinate *in vitro* and this agglutination correlated with stimulation by temperature and foreign bodies. Phagocytosis and agglutination of coelomocytes in the sea cucumber *A. japonicus* were correlated with different water temperatures and periods of incubation. A phagocytosis assay showed that spherocytes and amebocytes were able to phagocytose carmine and yeast, and the phagocytosis abilities were correlated with the kind and size of the foreign bodies. The peak phagocytosis percent and indices for carmine and yeast occurred at 18 °C. In the carmine phagocytosis assay, maximal phagocytosis (48%) occurred after 6 h, and phagocytosis was complete after 108 h. In the yeast phagocytosis assay, maximal phagocytosis (35%) occurred at 4 h, and was complete by 96 h (Li et al., 2009). Amebocytes are highly efficient at cleansing zymosan A. In fresh preparations, the petaloid pseudopodia appeared to collapse and redistribution of the cytoplasm produced branching filiform pseudopodia of considerable length, often intermeshing with the filiform pseudopodia of other amebocytes. The amebocytes clotted with morula cells and lymphocytes with increasing temperature and duration, which reduced the effectiveness of phagocytosis (Xing and Yang, 2009).

15.1.4 IMMUNE FACTORS

Many humoral immune factors exist in the coelomic fluid of sea cucumbers, which are able to recognize and attack foreign materials. Lectins play an important role by aggregating on exogenous cells to regulate trauma (Canicatti and Parrinello, 1985). Two kinds of Ca^{2+}-dependent agglutinins (C) were found in the coelomic fluid of *A. japonicus* by Matsui et al. (1994). C-mannose-binding lectin (MBL), which occurs in higher animals, was also found in the coelomic fluid (Eliseikina et al., 1999). It can be recognized by a variety of receptors on the cell membrane of marine bacteria, and shows high homology with vertebrates. It can be regarded as the origin of vertebrate MBLs (Mannan-binding

lectins), and has also been confirmed to be produced by mulberry cells. MBL may play an important role in activation of the complement system. In addition, two important complement components, AjC3 and AjC3-2 (Zhou et al., 2011), have been found within the coelomic fluid of the sea cucumber. The results of previous studies thus indicate the two complement components in sea cucumbers play a key role in the immune response during bacterial infection.

15.1.5 INFLUENCE OF ENVIRONMENTAL STRESS ON IMMUNOLOGICAL FUNCTIONS

A. japonicus live mainly in shallow coastal waters, and are thus subjected to changes in water temperature and salinity, and to other environmental stresses. Coastal aquaculture systems typically change water at a rate of 30–50% per day, meaning that the temperature can fluctuate by as much as 10 °C (Dong et al., 2007), and sea cucumbers may suffer adverse effects during transportation and production as a result of temperature changes and exposure to air.

Wang et al. (2008) found that heat stress significantly increased the phagocytic activity of coelomic fluid cells over a short period of time (0.5–3 h), but conversely, phagocytosis was not affected by hypothermia (Figure 15.6A). Superoxide dismutase and catalase activities in the coelomic fluid were reduced by hyperthermic stress (32 and 24 °C) (Figure 15.6B, C), while myeloperoxidase activity in the coelomic fluid increased under cooling stress, and decreased under heat stress (Figure 15.7A). Lysozyme activity also increased under heat stress (Figure 15.7B).

Experiments showed that relatively high salinities (25 and 35) increased phagocytosis over a short period of time (Figure 15.8A). Superoxide dismutase increased under both high and low salinity stresses (Figure 15.8B), while catalase activity decreased under low salinity stress (Figure 15.8C). Myeloperoxidase and lysozyme activities were unaffected by salinity.

15.2 SYMPTOMS AND TREATMENTS OF DISEASES

The rapid expansion and intensification of sea cucumber aquaculture has led to the occurrence of various diseases that are responsible for serious economic losses and have become one of the limiting factors in the sustainable development of this industry. However, research into diseases of cultured sea cucumbers in China is in its infancy, and little information is therefore available. We present the results of epidemiological studies that revealed several diseases reported currently.

15.2.1 DISEASES OF LARVAE

15.2.1.1 Rotting-edges syndrome

Epidemiology and etiology: Rotting-edges symptoms occur during the auricularia stages from June to July, and were prevalent in sea cucumber hatcheries in Penglai, Changdao, and Jiaonan in Shandong Province from 2003 to 2004, resulting in high mortality of up to 90% in certain cases. One dominant bacterial isolate was obtained from infected sea cucumber specimens through pathogenic analysis by Zhang et al. (2010a). It was used for artificial infection tests and was identified as *Vibrio lentus* through morphological, physiological, and biochemical analysis and 16S rDNA sequence analysis. The main sources of the pathogen include bottom feces, the water used to culture the larvae, and water that bathes the broodstock.

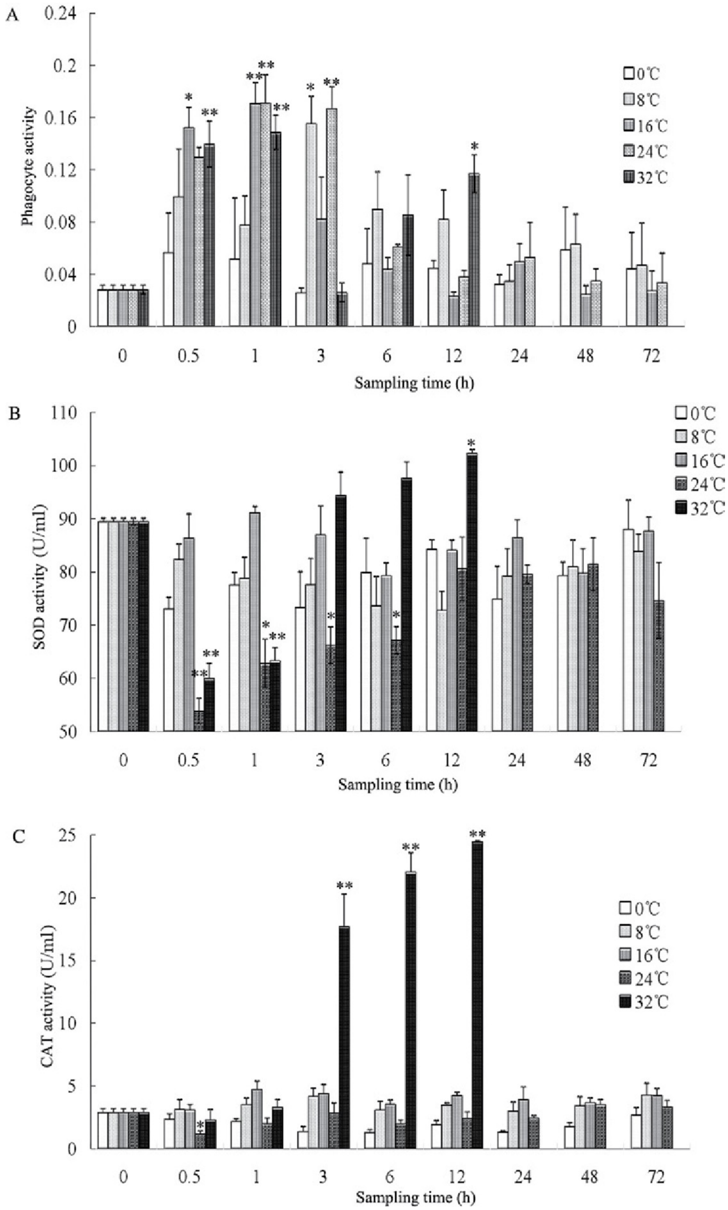

FIGURE 15.6

Effect of exposure to various temperatures on (A) coelomocyte phagocyte activity, (B) superoxide dismutase (SOD) activity, and (C) catalase (CAT) activity in coelomic fluid of *A. japonicus*. Data shown as mean ± SE, $n = 5$. Asterisks indicate significant differences: $*P < 0.05$, $**P < 0.01$.

From Wang et al., 2008

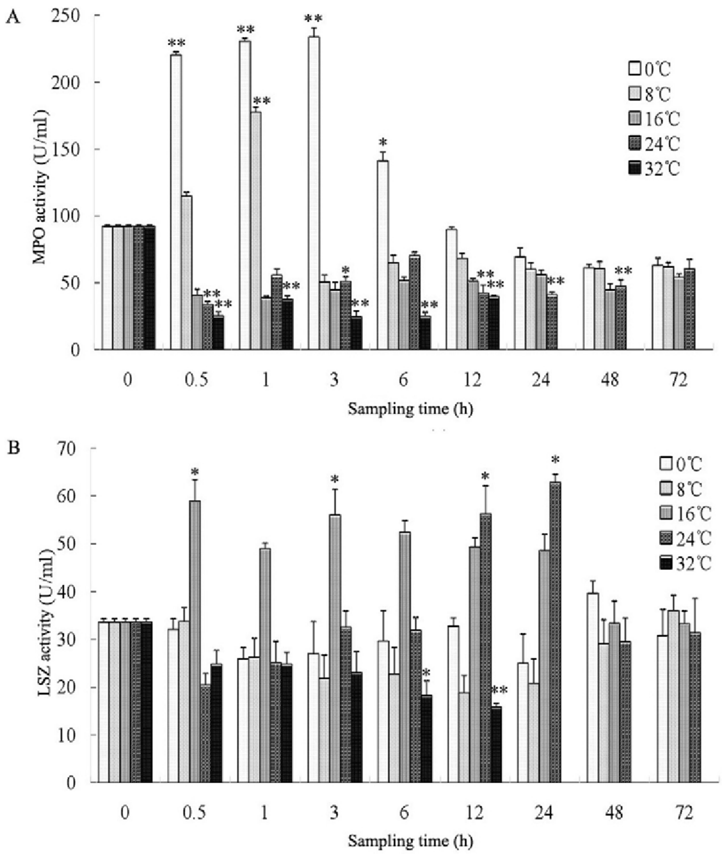

FIGURE 15.7

Effect of exposure to various temperatures on (A) myeloperoxidase (MPO) activity and (B) lysozyme (LZM) activity in coelomic fluid of *A. japonicus*. Data shown as mean ± SE, $n = 5$. Asterisks indicate significant differences: $*P < 0.05$, $**P < 0.01$.

From Wang et al., 2008

Clinical signs: The clinical signs in infected animals include thickening and darkening of the body edges at the auricularia stage. Diseased specimens undergo autolysis (Figure 15.9) and the body completely disintegrates within two days. Even if metamorphosis is in progress when the disease emerges, development will be delayed and the survival rate will be low. Most larvae will disappear from the settlement Plate one week post-settlement (Zhang et al., 2010a).

Histopathology: Infected larvae show dark purple edges when stained with hematoxylin and eosin. Numerous inflammatory cells appear under the epithelial cells between the larval ridges, infiltrating the spheroplast in some larvae. More seriously, the epithelial tissues become ulcerated with epithelial cell necrosis in severely infected individuals. No obvious pathological changes have been observed in the main internal organs of infected larvae, apart from shedding of gastric mucosal cells from the epithelial tissues in a few infected individuals (Wang et al., 2004).

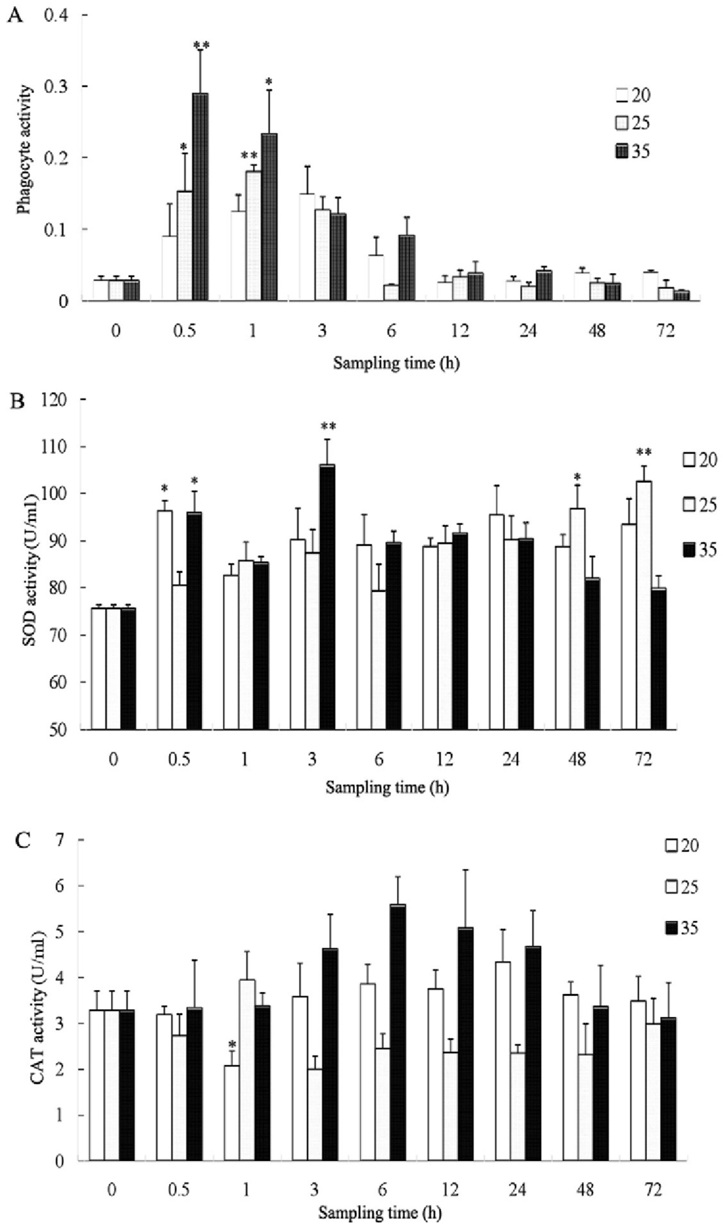

FIGURE 15.8

Salinity stress in *A. japonicus* relative to (A) coelomocyte phagocyte activity, (B) superoxide dismutase (SOD) activity, and (C) catalase (CAT) activity in coelomic fluid. Data shown as mean ± SE, $n = 5$. Asterisks indicate significant differences: *$P < 0.05$, **$P < 0.01$.

From Wang et al., 2008

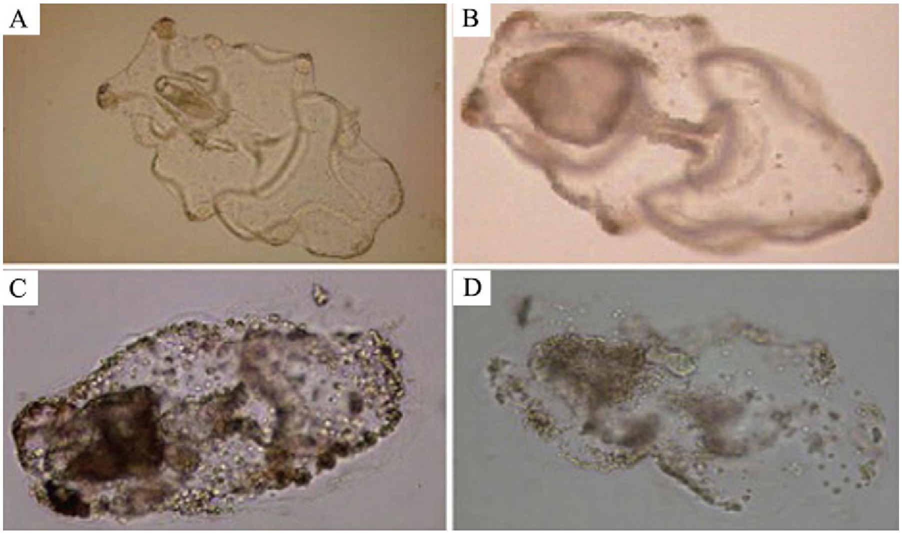

FIGURE 15.9

Autolysis process of *A. japonicus* larvae exhibiting rotting edges syndrome. (A) A normal auricularia. (B) Thickened and darkened edges at the early stages. (C) Edges of the larva decay and stomach shrinks as the condition develops. (D) The whole body disintegrates and autolyzes, and eventually the larva dies.

From Zhang et al., 2010a

Treatments: (1) Keep water clean in nursery pond, replace water regularly, and monitor water quality. (2) For prevention, tests showed that the pathogen could be controlled by furans antibiotics using 3×10^{-6} units in the whole pond during two periods of three consecutive days. For treatment, the drug is used at a higher concentration of 5×10^{-6} units once a day until larvae are healed (Zhang, 2004).

15.2.1.2 Stomach ulcer disease

Epidemiology and etiology: This disease typically occurs from June to July when the temperature and stocking densities are high. It is observed along the coastal area of Shandong and Liaoning provinces, with a high mortality of 90%. Generally, some auriculariae show shrunken stomachs 5–6 days after hatching when the water temperature is between 15 and 23 °C. The late auricularia stage (7–10 days posthatching) and the first part of metamorphosis (from auricularia to doliolaria) are the periods of peak disease incidence. The disease spreads rapidly, causing larvae to die of stomach ulceration within one week. Surviving individuals develop slowly and remain weak after larval settlement. Several researchers have suggested that the outbreak of this disease is associated with high-stocking densities and the supply of aging, low-quality, poorly nutritive microalgae (Wang et al., 2004, 2006b; Yu et al., 2005). Traditional physiological and biochemical methods, as well as semiautomatic identification using an API system, were applied for the bacterial classification of this disease by Wang et al. (2006b); *Vibrio splendidus* was identified as the causal agent, a species regarded as one of the major pathogens causing stomach ulcer disease. However, another dominant bacterial strain was isolated from affected auricularia and was also verified to cause stomach ulcer disease using an artificial infection test, indicating that the causative pathogens are complicated and diverse.

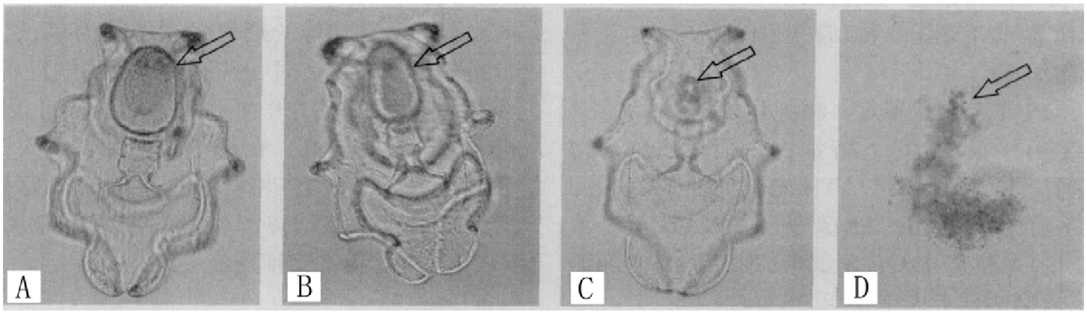

FIGURE 15.10

Pathogenesis of stomach ulcer disease in *A. japonicus* auricularia. (A) Normal auricularia (arrow). (B) Stomach wall becoming thick and rough (arrow). (C) Shrinkage of the stomach (arrow). (D) Body lysis (arrow).

From Wang et al., 2006b

Clinical signs: The stomach walls of larvae are thick, rough, and poorly defined. The pear-like stomach of the normal auricularia gradually shrinks and deforms, and then spreads over the surface of the body, with infected larvae ultimately dying (Figure 15.10, Wang et al., 2006b). High-resolution microscopy revealed active bacteria with short rods and a single polar flagellum present in the stomach walls. Stomach ulceration usually results in starvation, delayed growth and development, abnormal morphology and size, and a relatively low rate of metamorphosis from the auricularia to the doliolaria stage.

Histopathology: The pear-shaped stomach in the normal auricularia consists of an integrated and simple columnar epithelium. Obvious pathological changes were observed in the stomach epithelium in infected larvae, appearing hyperplastic, thickened, and heavily stained, resulting in necrosis and shedding of the stomach epithelium. Moreover, numerous bacteria were observed in the stomach and its walls. Meanwhile, esophageal sphincter cells were infiltrated by inflammatory cells. The shrunken stomach became necrotic in severely infected larvae (Figure 15.11, Wang et al., 2006b).

Treatments: (1) Using only fresh and palatable phytoplanktonic cells, such as *Chaetoceros*, *Dunaliella*, or marine yeast as food. (2) Increasing water exchange, reducing the number of bacterial count in the water, and adding furans antibiotics ($3 \times 10^{-6}\sim$ to 5×10^{-6} units) could have a remarkable curative effect on stomach ulcer disease (Rong, 2005).

15.2.1.3 Gas bubble syndrome

Epidemiology and etiology: This condition has only been detected in the auricularia stage and is associated with relatively low mortality. Some studies have suggested that excess aeration in the rearing tanks can lead larvae to swallow microscopic gas bubbles (Zhang and Liu, 1998).

Clinical signs: The presence of gas bubbles inside the larva causes reduced food intake, leading to death in the most severe cases.

Treatments: Moderate intensity and intermittent aeration should be used (30 min every 2 h) to decrease the incidence of gas bubble syndrome.

15.2.1.4 Off-plate syndrome

Epidemiology and etiology: This condition is also known as “adhesion dysfunction disease” and “disintegration disease,” and occurs in larvae that have settled after completion of metamorphosis from the

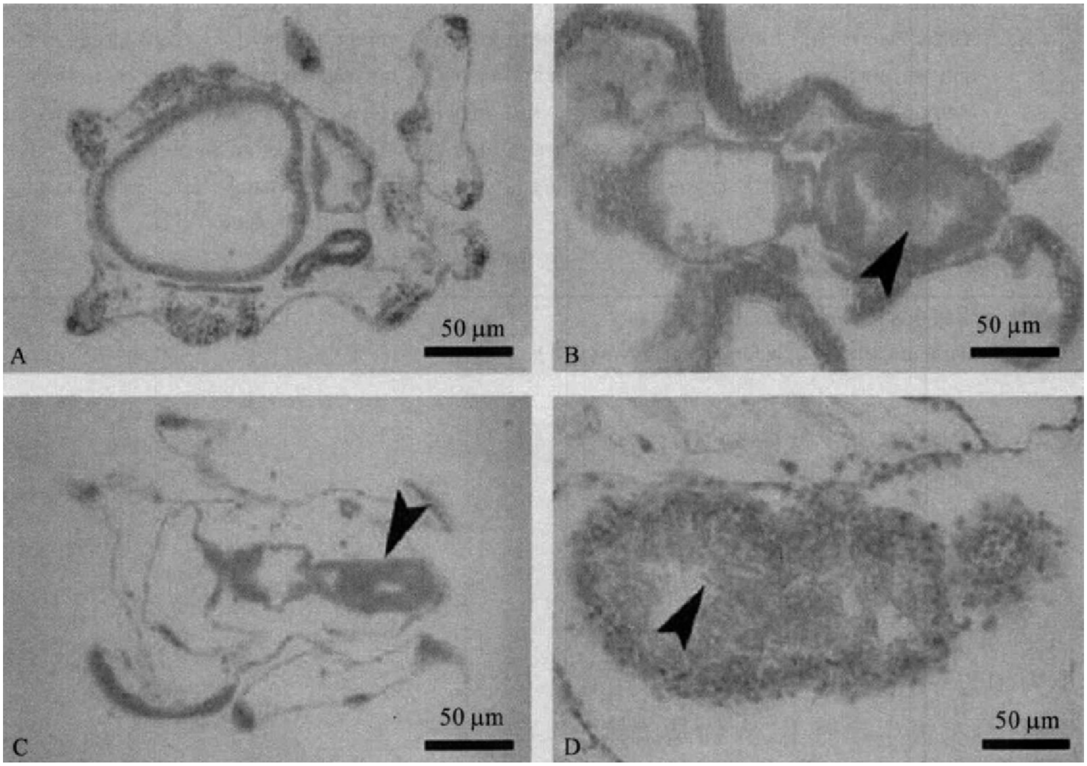

FIGURE 15.11

Histological changes in *A. japonicus* auricularia exhibiting stomach ulcer disease. (A) Pear-like stomach of normal auricularia (arrow). (B) Stomach epithelium appears hyperplastic and thickened, epithelium cells necrotic and shedding off (arrow). (C) Shrunken stomach becomes small (arrow). (D) Stomach epithelium hyperplastic, nucleus swollen and heavily stained (arrow).

From Wang et al., 2006b

doliolaria to the pentactula stage. It is a serious disease, which is prevalent during the late period of larval rearing. It is characterized by severe infection, rapid spread, and high mortality (to 100% within several days). Zhang (2004) reported that off-plate syndrome was caused by several bacteria, and three dominant bacterial strains, coined HB-1, HB-2, and HB-3, have been isolated from specimens collected from different hatcheries. Analysis using morphological, physiological, biochemical, and molecular biological methods demonstrated that off-plate syndrome was associated with the use of the algae *Sargassum thumbergii*, though another dominant bacterial strain has also been isolated from infected larvae (Zhang et al., 2009). Artificial infection demonstrated its role as a causative pathogen associated with this disease, and artificially infected sea cucumbers showed the same symptoms as naturally infected ones. This bacterium was identified as *Vibrio* sp. (Figure 15.12).

Clinical signs: Retracted arms and decreased activity were observed in infected larvae. Meanwhile, the epidermis of infected individuals showed brown spots. Some were surrounded by a transparent

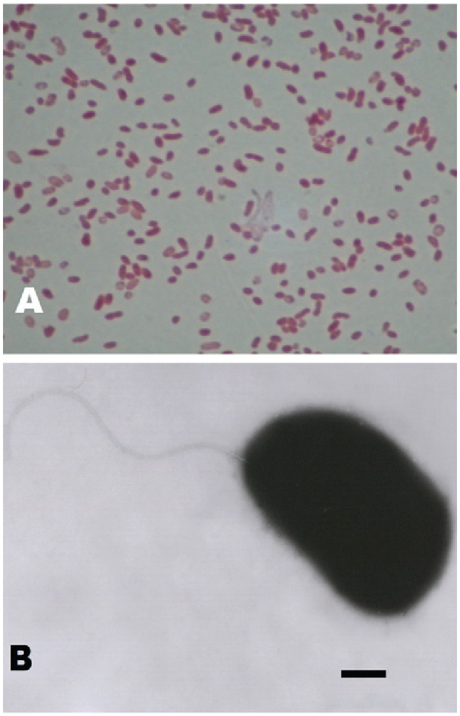

FIGURE 15.12

Off-plate syndrome in *A. japonicus.* (A) Gram-negative HB-1 bacteria staining. (B) Electron microscope observation. Scale bar = 500 nm.

From Zhang et al., 2009

membrane. Early ulceration usually occurs on the skin, leading to the disintegration of the whole body. In such cases, ossicles can be found on the bottom of the tanks as they fall from the degrading bodies.

Histopathology: The obvious pathological changes observed in diseased larvae involved nuclear condensation, enlargement, and necrosis of epithelial cells in papillae. Symptoms include shedding of the cuticle layer of the epidermis and histolysis, resulting in the shrinkage and blunted appearance of papillae with shrinkage of digestive organs and necrosis. The ventral tube foot, tentacles (arms), and surface epithelial tissues of the body wall are susceptible to infection. Synergistic effects of internal (pathogens in diet species) and external (pathogens on the substrate) pathogenic factors thus contribute to larval infection.

Treatments: (1) Using two stages of sand filtration or ultraviolet disinfection; clearing unconsumed food, feces, organic matter, etc., from the bottom of the tanks; and cleaning tanks timely can minimize the number of bacteria in culture water. (2) Paying attention to the quality and quantity of foods, disinfecting sea mud and other food (e.g., *Sargassum*), to eliminate infectious agent. These measures should be combined with regular microscopic examination to observe feeding activity and physical conditions of the larvae. If the disease occurs, the use of an antibacterial agent like quinolone in the tanks or pond, and/or with the food is recommended (Rong, 2005).

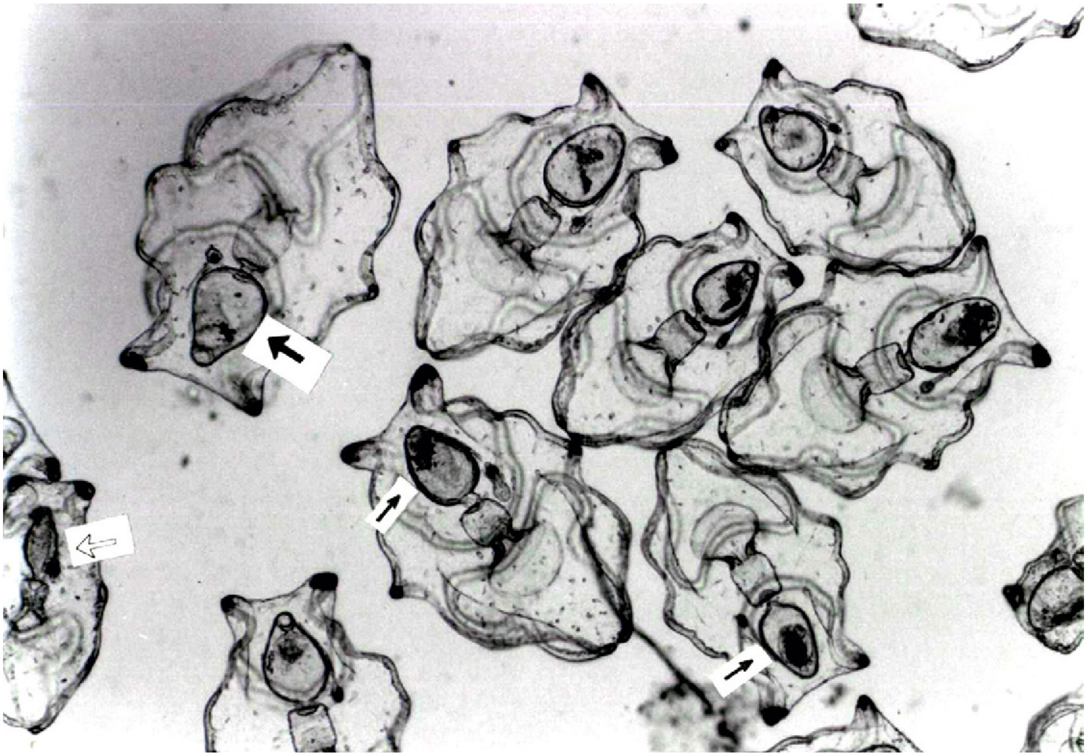

FIGURE 15.13

Comparison of normal larvae and larvae exhibiting stomach atrophy syndrome in *A. japonicus.* The stomach of normal larvae is pear-shaped with thin walls (black arrow), whereas that of diseased larvae is shrunken with thicker walls (white arrow).

From Deng et al., 2008

15.2.1.5 Stomach atrophy syndrome

Epidemiology and etiology: In 2006, an epidemic condition coined stomach atrophy syndrome was detected in the sea cucumber *A. japonicus* and was prevalent in Liaoning Province (China). The area of influence was wide and the associated mortality was high. This epidemic occurred mainly in larvae at 5–7 days posthatching. Preliminary indications pointed to a viral etiology (Deng et al., 2008).

Clinical signs: The stomach of diseased larvae shrank gradually and the stomach wall tended to appear thick, rough, and distorted until the stomach became small and round (Figure 15.13). This was accompanied by starvation, delayed growth and development, and a relatively low rate of early metamorphosis from the auricularia to the doliolaria stage. In some cases, metamorphosis had not taken place even after 20 days of pelagic life.

Histopathology: Examination of ultrathin histological sections of larvae by electron microscopy revealed that a virus mainly affects the cytoplasm in connective tissue and epithelial cells, especially the microvilli (Figure 15.14A) and the membrane of epithelial cells (Figure 15.14B). The mature virions

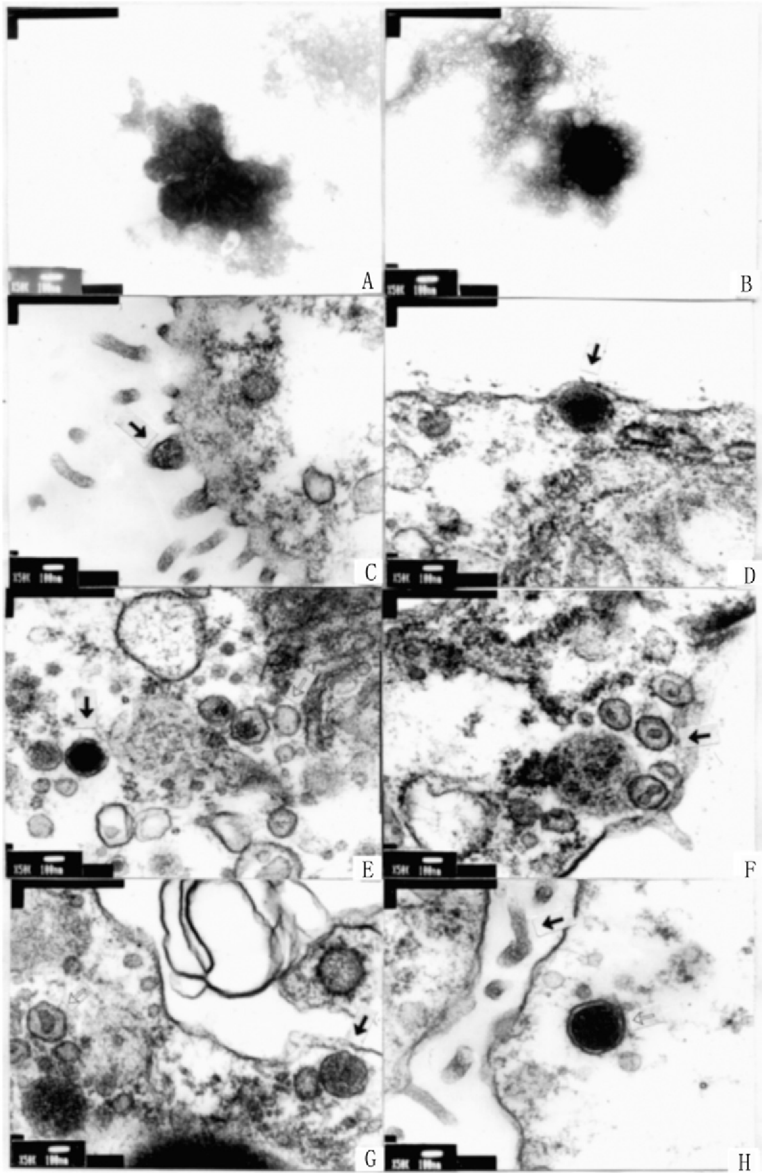

FIGURE 15.14

Stomach atrophy syndrome in *A. japonicus*: observation of virions by negative staining of ultrathin sections. (A) Five naked virions on homogenate sample by negative staining, ×50,000. (B) Photographs of negatively stained virus of the parents, ×50,000. (C) A mature virion that was moving toward the cell, passing through the microvilli and being released outwards (arrow), ×50,000. (D) A spherical virion was passing through the nearly degraded epithelial cell membrane and being released outward (arrow), ×50,000. (E) A mature spherical virion in the apoptotic cell (black arrow); an empty virion in the apoptotic cell (white arrow), ×50,000. (F) The nuclear-shaped virions in the cell (black arrow), ×50,000. (G) The naked virions in the disintegrated stomach epithelium cell (black arrow), assembling hexagonal virions (white arrow), ×50,000. (H) A mature virion (white arrow) with envelope in the nearly empty cell; shed microvilli of epithelial cells (black arrow), ×50,000.

From Deng et al., 2008

are dispersed in the cytoplasm and appear approximately spherical or hexagonal with double inner envelopes (Figure 15.14C–E). Invasion and proliferation of the virions disrupt the cells and organelles, leading to cytoclasis (Figure 15.14F).

Treatments: There are no known effective methods to control the disease.

15.2.2 DISEASES OF JUVENILES

15.2.2.1 Bacterial ulcer disease

Epidemiology and etiology: Juveniles smaller than 5 mm are susceptible to this infection, which tends to occur as a result of high temperatures and stocking densities. The infection spreads rapidly from diseased individuals to healthy ones, making it difficult to control. Occasionally, an entire population can be wiped out in a short time, once the infection sets in. Bacterial infection has been reported to be the main cause of the disease, though the characteristics of the bacteria have not been described. The causative agent proliferated on PVC plates, appearing as red, pink, or purple-red patches. Infected juveniles die and disintegrate (Zhang and Liu, 1998).

Clinical signs: Infected individuals are weak and not able to feed; their bodies shrink and eventually adopt a rounded shape and become white. Skin ulceration begins with the appearance of small white patches that enlarge, and eventually expose the underlying muscle and ossicles. Finally, the whole body disintegrates and only white dots are clearly visible in the substrate.

Treatments: After using terramycin or nitrofurazone ($3 \times 10^{-6} \sim 5 \times 10^{-6}$ units) for a duration of two or three days, the disease should be controlled and future occurrences prevented effectively (Zhang and Liu, 1998).

15.2.2.2 Scuticociliatida

Epidemiology and etiology: This disease typically occurs in summer as a result of high temperatures (above 20 °C). Juveniles are susceptible to this disease 2–3 days after settlement. The disease is prevalent in Penglai, Changdao in Shandong Province (China), and shows characteristic wide epidemicity, infectivity, and high mortality rate in a short time. However, no case has been observed in the larval stage. Morphological studies have been made on living specimens treated with Chatton–Lwoff silver impregnation techniques, as well as by scanning electron microscopy. The results showed that the pathogen belongs to ciliates in the order Scuticociliatida, but precise identification remains unclear. Melon-seed-shaped living ciliates have a thin membrane with a full size around $38.4 \times 21.7\,\mu m$. An initial bacterial infection made the juvenile weak, facilitating infestation by the ciliates that caused death (Rong, 2005).

Clinical signs: The juveniles have lesions on their body wall and become weak (after infestation by ciliates). Growing invasion into the tissues and proliferation of ciliates induces disintegration of the tissues.

Treatments: (l) Water should be treated with sand and filtrated on 300 μm silk mesh. (2) Removing the deposited material lying on the bottom of the tanks or ponds, washing settlement plates frequently, and transferring the juveniles from a pond to another pond timely. (3) Before feeding, food should be treated with drugs to control the presence of pathogenic bacteria or ciliate. (4) Appropriate antibiotics like dithiocyano-methane can be used to keep sea cucumber larvae healthy before settlement, as they will be more resistant to ciliates at the juvenile stage (Rong, 2005).

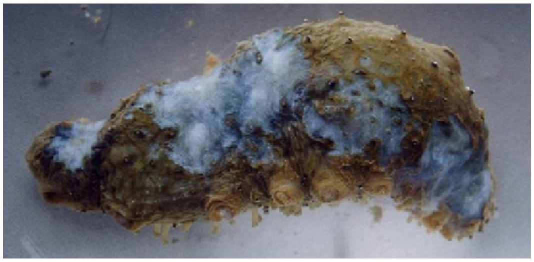

FIGURE 15.15

Young sea cucumber *A. japonicus* exhibiting the skin ulceration syndrome.

From Wang et al., 2006a

15.2.3 DISEASES OF YOUNG AND ADULTS

15.2.3.1 Skin ulceration syndrome

Epidemiology and etiology: This is also known as "ulcer disease" of the skin or "dissolved body disease," and has been the most common and serious disease in recent years. It occurs all year round, but especially in spring, when the ice begins to melt. Both young and adults of *A. japonicus* can be infected, though mortality, morbidity, and infectivity are higher in young sea cucumbers compared to adults. The disease has a high mortality rate of up to 90% and spreads rapidly. There is currently no consensus regarding the responsible pathogen. Zhang et al. (2006) suggested that *Vibrio splendidus* was responsible in Qingdao in Shandong Province (China), while Wang et al. (2006a) identified *Pseudoalteromonas nigrifaciens* as the pathogen in Shandong and Liaoning provinces (China). Yang et al. (2007a, 2007b) considered that sea cucumbers with length from 4–8 cm were infected by *Vibrio alginolyticusa* in Rushan in Shandong Province, and Wang et al. (2007a) identified *Aeromonas salmonicida masoucida* as the pathogen in Rizhao in Shandong Province, and *Aeromonas medin* in Yantai in Shandong Province.

Clinical signs: Infected sea cucumbers shrink and small lesions appear around the mouth, while the tentacles are black and unable to contract or close. The viscera of most sea cucumbers are usually expelled as the infection progresses. In the middle stage of infection, the bodies of the infected individuals are rigid and discolored. The tube feet shrink, becoming flat and white with no ability to attach to the substrate. Early ulceration usually occurs around the mouth and ventral surfaces, forming a bluish-white spot. The infected skin becomes eroded with deep ulcerations and the lesions gradually expand, with increased mucous synthesis over large areas of the body wall. Infected sea cucumbers finally die, becoming deliquescent when touched (Figure 15.15, Wang et al., 2006a).

Treatments: (1) Ensuring appropriate density of juveniles, as well as good water and sediment quality. (2) Applying a treatment in the fall to prevent the appearance of disease in winter. At the onset of winter, addition of chlorine dioxide and povidone-iodine can be used to oxidize the bottom organic matter and kill pathogens, respectively. Once the sea cucumbers resume feeding, feeding them with specialized aminoglycoside antibiotics should allow sea cucumbers to accumulate a certain concentration of drugs to overwinter safely. (3) Monitoring activity level, body wall changes, feeding and defecation rhythms, and sanitary condition of the bottom of the pond. Measuring the quality of water and growth rate of sea cucumbers regularly is also a prerequisite to early detection of the disease. Individuals whose body is badly affected should be removed and buried. Healthy or mildly infected

individuals should be treated with aminoglycosides in the water and simultaneously fed with them. (4) When water heaters or groundwater are available, raising the temperature above 14 °C improves feeding and disease resistance (Rong, 2005).

15.2.3.2 Fungal diseases

Epidemiology and etiology: Fungal diseases frequently occur in pond-cultures from April to August. Both young and adults can be infected by fungi, but no case of fungal infection has been found during the larval stages. Although this type of disease does not cause massive death, it results in an unhealthy appearance and poor quality of the final product. Through microscopic examination and cultivation of infected individuals, Zhang et al. (2010b) identified fungi as the main pathogen, as abundant mold spores and mycelium were observed in the lesions. It is likely that fungal proliferation was promoted by excessive quantities of organic matter or decaying macroalgae, as well as by a deficit in dissolved oxygen concentration.

Clinical signs: The body appears bluish white with excoriation and exposure of the muscle layer (Zhang et al., 2010b). Edema and rotten epidermis may also be apparent. In some cases, the whole body surface becomes discolored and transparent, and the body wall in swollen individuals becomes thinner, and soft when touched. The papillae of infected sea cucumbers become white during the early stage of infection, and the lesions gradually enlarge. Severe infection can lead the papillae to rot or become covered with white patches. As the infection develops, large areas of the body wall appear bluish white as the skin becomes eroded and the underlying tissues are exposed. Finally, the exposed skin appears rigid and dry.

Treatments: (l) Avoiding excessive feeding, keeping the bottom and the water clean. (2) Avoiding proliferation of green algae (macrophytes), and keeping the bottom clear of algal debris to prevent environmental degradation. (3) Cleaning up and drying the pond every week to prevent excessive accumulation of organic matter (Rong, 2005).

15.2.3.3 Acute peristome edema disease

Epidemiology and etiology: Studies have shown that this disease only occurs in the overwintering stage of *A. japonicus* and that infected sea cucumbers faced potentially high mortality rates (>90%). In Dalian, where frequent disease outbreaks occurred from the beginning of February to March, infected sea cucumbers died seven days after the first clinical signs appeared (Wang et al., 2005, 2007b). Observations in electron microscopy demonstrated virus-like particles without inclusion bodies located in the different tissues and concentrated in tentacular tissue during the high-incidence season.

Clinical signs: This disease is characterized by skin ulceration and the presence of huge amounts of viscous mucous. Individuals lose sensitivity to outer stimulation. Ulceration usually occurs around the mouth then spreads rapidly over the whole body. The tentacles and mouth swell, preventing complete tentacular retraction. The intestine is usually expelled as the infection develops. In severe cases, the body wall becomes misshapen and shed ossicles are found at the bottom of the rearing tanks. The body wall eventually dissolves into a mucous-like colloid until the infected specimen dies.

Histopathology: Numerous vacuoles formed by the disintegration of organelles can be observed in cytopathic cells infected by the virus. Cytopathic changes include breakage of the outer membrane and disintegration of swollen endoplasmic reticula, with blurred limits. Many hyperplastic viral cores are scattered around the endoplasmic reticulum, and abnormal structures, such as medullary loops, appear as a result of the disintegration of injured mitochondria (Figures 15.16–15.18) (Wang et al., 2005, 2007b).

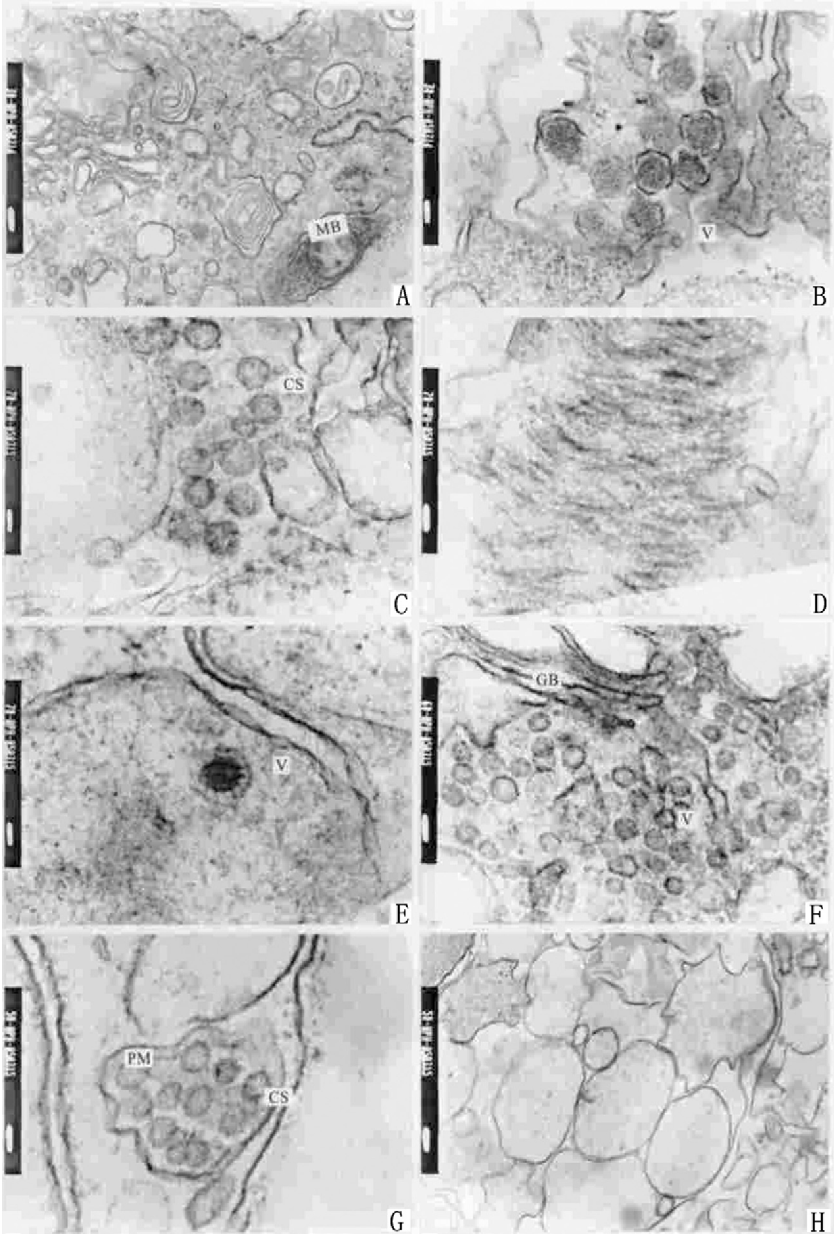

FIGURE 15.16

Acute peristome edema disease in *A. japonicus.* (A) Myeloid body and mass empty vesicles in cells of tentacle (left border scale bar = 200 nm). (B) Mature virus with plasma membrane enveloped in cells of tentacle (scale bar = 100 nm). (C) Core structures of virus scattered in cells of hemal vessel (scale bar = 50 nm). (D) Disordered fiber structures in cells of hemal vessel (scale bar = 100 nm). (E) A virion being assembled in cells of respiratory tree (scale bar = 50 nm). (F) Golgi body and Golgi vesicles in cells of respiratory tree (scale bar = 100 nm). (G) Mature virus with plasma membrane enveloped in cells of papillate podium (scale bar = 50 nm). (H) Vacuolar structures in cells of papillate podium (scale bar = 200 nm). CS, core structures; MB, myeloid body; Mi, mitochondrion; PM, plasma membrane; rER, rough endoplasmic reticulum; V, virus.

From Wang et al., 2005

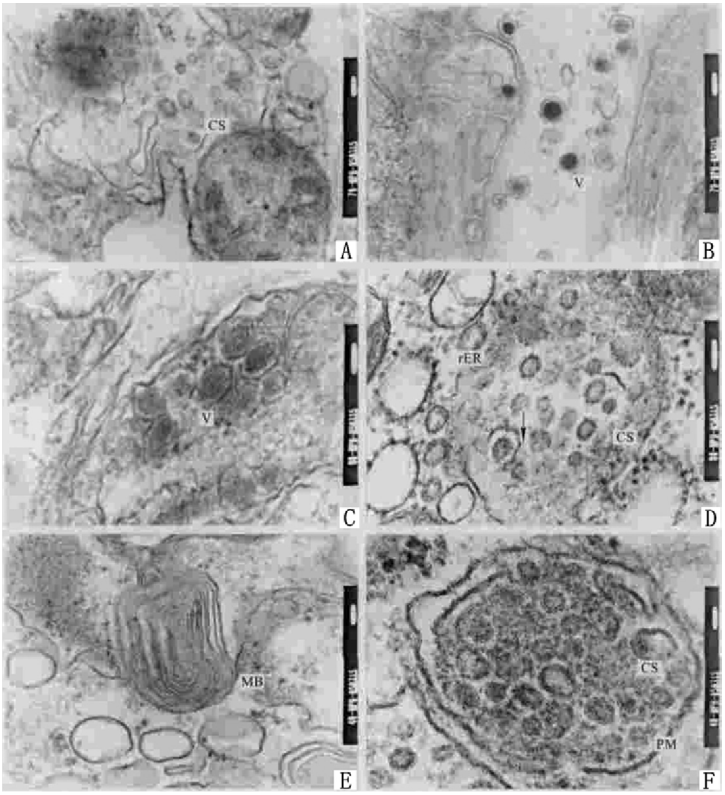

FIGURE 15.17

Acute peristome edema disease in *A. japonicus.* (A) Clustered virus coated by plasma membrane in cells of hemal vessel (left border scale bar = 100 nm). (B) Mature virus scattered in cells of hemal vessel (scale bar = 200 nm). (C) Mature virus with membrane in cells of intestines (scale bar = 100 nm). (D) Core structures of virus and virus being enveloped (black arrow) (scale bar = 100 nm). (E) Myeloid body and vacuolar structures in cells of tentacle (scale bar = 100 nm). (F) Core structures of virus with plasma membrane coated in cells of tentacle (scale bar = 50 nm). CS, core structures; GB, Golgi body; MB, myeloid body; Mi, mitochondrion; PM, plasma membrane; rER, rough endoplasmic reticulum; V, virus.

From Wang et al., 2005

Treatments: Norfloxacin and ofloxacin (fluoroquinolones) have a strong inhibitory effect on the pathogen; they can be used as treatment when applied during the early stage of the disease (Ma et al., 2006).

15.2.4 PARASITIC DISEASES

15.2.4.1 Protozoan infection

Epidemiology and etiology: *A. japonicus* is susceptible to protozoal infection during winter. Infection occurs in young individuals and adults, and does not generally cause serious mortality. Histology revealed infusorians attached only to the respiratory tree, most often in great numbers in the inner

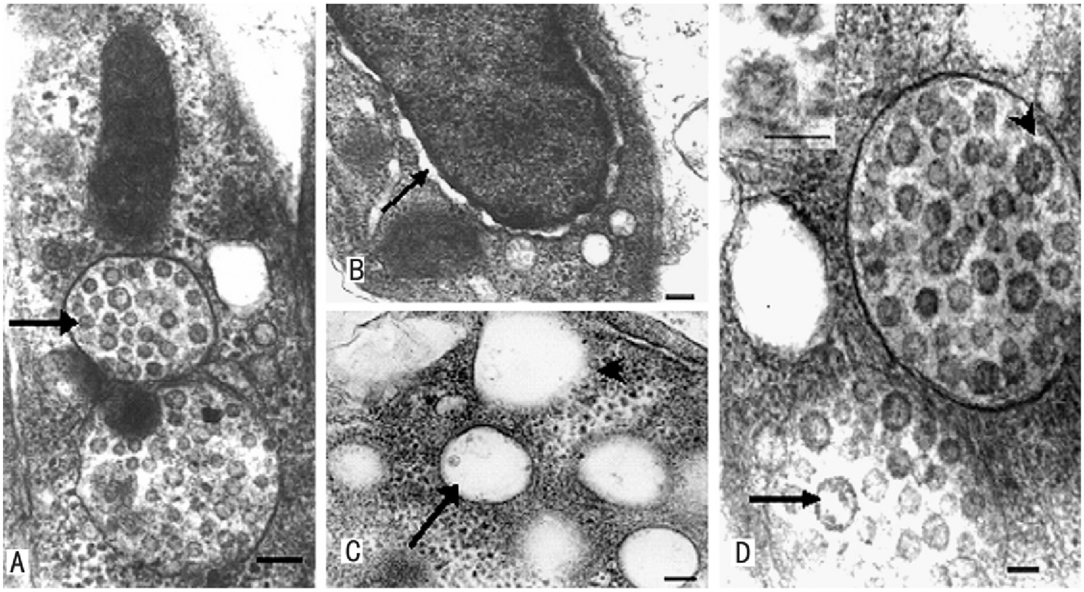

FIGURE 15.18

Pathologic changes in cells of intestinal epithelium of *A. japonicus* with acute peristome edema disease. (A) A cytopathic cell with two vesicles full of virions (arrow) (scale bar = 200 nm). (B) A nucleus of virus-infected cell was of homogenous materials and hyperstained, with no nucleoli observed, and the endoplasmic reticulum obviously swelled and formed a separation cisternae (arrow) between the nucleus and the cytoplasm (scale bar = 200 nm). (C) Empty vesicles dispersed in granular areas of cytopathic cell of intestine epithelium, some with clear membrane (arrow), and some with blurry margins (arrowhead) (scale bar = 200 nm). (D) Virus containing vesicles in cytopathic cells of the intestine, in which some are light-colored in the center (arrow) as if they are hollow and some are overcolored with petal-like pattern (arrowhead). Orderly tubular structures can also be found around the vesicles (scale bar = 50 nm). Inset: a typical virus particle, with an envelope and regular petal-like surface projections (arrow) surrounding the periphery of the particle (scale bar = 100 nm).

From Wang et al., 2007b

wall of the branches. The head of the parasite penetrates through the epithelial tissues of the inner wall, causing tissue damage and ulceration, and even evisceration in severe infections. According to morphological studies on living specimens treated with Chatton–Lwoff silver impregnation techniques and scanning electron microscopy, the parasite is a type of ciliate (*Boveria* sp.). The polypeptide is 40–75 μm long and 20–27 μm wide, but its species name is unknown (Rong, 2005).

Clinical signs: Compared with normal animals, infected individuals show no conspicuous lesions, though the intestine and respiratory tree may be eviscerated following severe infection. After evisceration, individuals tend to be thin, weak, and sluggish as a result of feeding disturbances, which make the animals susceptible to secondary infections.

Treatments: No information available.

15.2.4.2 Platyhelminthes infection

Epidemiology and etiology: This type of disease is prevalent from January to March when the water temperature is low (<8 °C), and occurs in young and adults, causing high mortality rates among cultivated sea cucumbers. The symptoms are relieved or disappear when the water temperature rises above 14 °C. Examination of specimens revealed that both flatworms and bacteria were associated with this disease; flatworms invaded animals that were already infected by bacteria, thus aggravating the illness and accelerating death in *A. japonicus*. Flatworms are thus considered to be one of the pathogens of skin ulceration syndrome, as a secondary infection (Wang et al., 2004). Flatworms are slender and straight, with varying lengths and a polymorphic body form. This parasite has only been found in *A. japonicus* to date, and is thus referred to as the *A. japonicus* flatworm.

Clinical signs: The symptoms of this disease are similar to those of skin ulceration syndrome. Ulcers develop on the dorsal and ventral surfaces of the body, and severe ulceration can cause the outer tissues to rot, exposing the inner body tissues. Histological observations demonstrated that many flatworms were able to cause infected young sea cucumbers to lose their capacity to attach to the substrate. Dissection of infected individuals revealed that most were eviscerated and had lost the ability to feed.

Treatments: See treatments for skin ulceration syndrome.

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CHAPTER

AQUACULTURE, STOCK ENHANCEMENT, AND RESTOCKING

16

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SUMMARY

The production value of *Apostichopus japonicus* aquaculture surpasses that of all other marine species cultivated in China. This chapter presents the various production modes, including industrial (indoor) culture, pond (cofferdam) culture, suspended culture, and sea ranching of *A. japonicus*. Different facilities, production systems, techniques, and management schemes are outlined. New technologies applicable to industrial culture and novel systems developed in recent years for the culture of *A. japonicus* in ponds, and for stock enhancement in near-shore and off-shore areas, are also discussed. This chapter provides general guidelines and can hopefully be used as a reference for researchers or managers working on the culture and protection of *A. japonicus* and other sea cucumber species.

Keywords: *Apostichopus japonicus*; aquaculture; pond culture; restocking; sea cucumber; sea ranching; stock enhancement; suspended culture

16.1 INTRODUCTION

Apostichopus japonicus is a common temperate species of sea cucumber found in the northwest Pacific (Liao, 1980; Sloan, 1984) (see Chapter 3 for details on its geographic distribution). The demand for sea cucumber, in particular for *A. japonicus*, is increasing along with economic development and the improvement of living standards in China. It has resulted in rapid overfishing and depletion of natural stocks over the last 20 years (see Chapter 5). Consequently, natural populations of *A. japonicus* and other sea cucumbers are now dwindling, and the prices of dried and fresh products have increased sharply over the past decade. Concurrently, the *A. japonicus* industry also developed rapidly as a result of breakthroughs in captive breeding and culture techniques, currently providing a steady, high-quality supply of *A. japonicus* products to Chinese and international markets (see Chapters 21 and 22).

The *A. japonicus* industry has now overtaken the traditional shrimp and fish aquaculture industries (Fisheries Bureau of Ministry of Agriculture, 2006–2013); today, *A. japonicus* generates the highest single-species output value and profit in China. From 2005 to 2012, the culture area dedicated to *A. japonicus* has increased by 145.4% (Figure 16.1A) and total output by 161.7% (Figure 16.1B).

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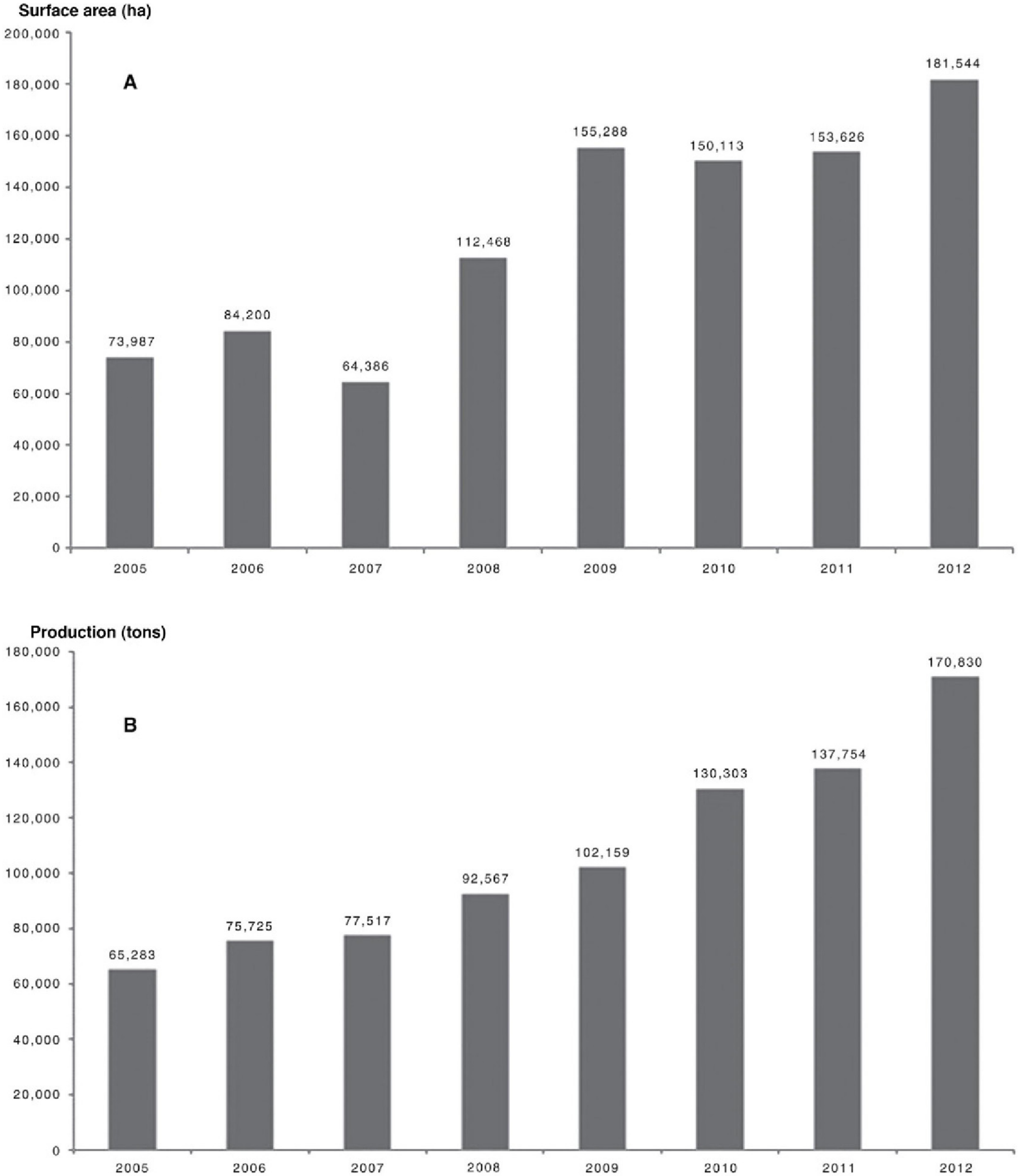

FIGURE 16.1

(A) Surface area and (B) production dedicated to the culture of *A. japonicus* in China between 2005 and 2012.

Data from Fisheries Bureau of Ministry of Agriculture, 2006–2013, and graphs by Libin Zhang

However, because *A. japonicus* require excellent seawater conditions to be successfully cultured, the facilities are located mainly around coastal regions in the natural native distribution range of the species, with the regions of Shandong and Dalian contributing the greatest output values (Fisheries Bureau of Ministry of Agriculture, 2006–2013).

The 2013 China Fishery Statistical Yearbook shows that, in 2012, the area occupied by *A. japonicus* culture was 181,544 ha for a total output that reached 170,830 tons, in an overall marine aquaculture sector that occupied 2,180,927 hectares and produced 16,438,105 tons. The output value of *A. japonicus* was up to 34.166 billion Yuan (about 5.647 billion USD) considering 200 Yuan (about 33.06 USD) as the average price per kilo. Although the share of *A. japonicus* culture area and output is barely 8.32 and 1.04%, respectively, its output value accounts for 16.09% of nationwide marine aquaculture. The Shandong and Liaoning provinces are the major producing areas. In 2012, *A. japonicus* cultures in Shandong occupied 62,673 ha and produced 82,905 tons (48.53% of the nationwide output), and Liaoning was at 105,903 ha and 64,512 tons (37.76% of the nationwide output) (Figure 16.2) (Fisheries Bureau of Ministry of Agriculture, 2006–2013).

At present, the main culture methods include indoor facilities, outdoor culture in ponds, and cofferdams and sea ranching. The indoor and pond techniques are well mastered and play an important role in the development of the *A. japonicus* aquaculture industry. However, these culture methods have negative impacts, including pollution and other disturbances that may compromise the quality of the final product. Cofferdam culture and sea ranching constitute healthier production modes associated with a more sustainable development. The environments where the culture is performed are closer to native conditions required by the species, and natural algae are available as food (see Chapter 10 for more information on feeding); cost of input is therefore considerably reduced. Other advantages of these two culture methods include reduced incidence of disease, faster growth rate, and better quality of *A. japonicus* products. End products obtained from cofferdam culture or sea

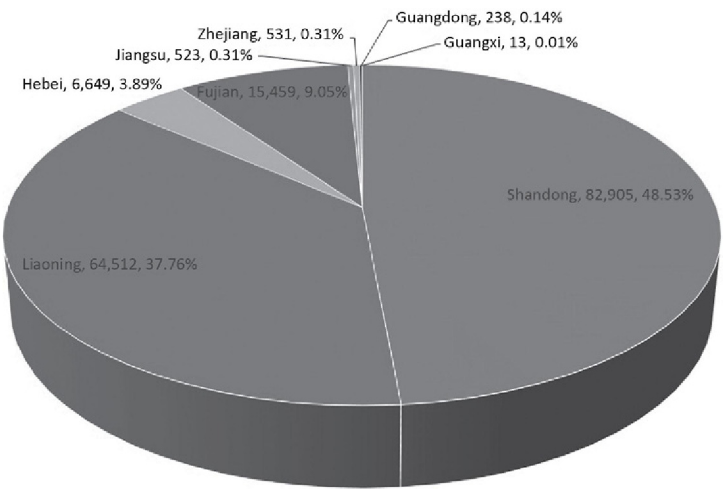

FIGURE 16.2

Production (tons) of *A. japonicus* in the main provinces of China in 2012.

Data from Fisheries Bureau of Ministry of Agriculture, 2006–2013, and graphs by Libin Zhang

ranching fetch prices that are 20–50% higher than indoor or pond cultivated products, and are more competitive in the marketplace. In addition, *A. japonicus* cultured in this manner can enhance benthic communities through their deposit-feeding activities. Therefore, these two culture methods have huge potential and broad prospects.

16.2 INDUSTRIAL CULTURE

The industrial culture of *A. japonicus* is a process that involves broodstock conditioning, artificial spawning induction, and larval culture. It was developed to provide adequate seedlings for pond culture and sea ranching.

In China, the *A. japonicus* industry is concentrated in Yantai and Weihai in Shandong Province as well as Liaodong Bay in Liaoning Province. Broodstock of *A. japonicus* is usually collected from the wild by local diving fishermen, and the most robust of the broodstock will be placed in culture ponds for temporary rearing. These broodstocks will experience nutrient fortification through artificial feeding for months and be subjected to artificial spawning thereafter (see Chapter 7). Artificial fertilization and larval culture will follow. After metamorphosis, juveniles will be distributed into different nursery tanks. Then, the juveniles will undergo intermediate rearing indoor or in ponds. The resulting seedlings can be sold after an appropriate time that will depend on the users' need; these juveniles will undergo final grow out following various methods.

16.2.1 FACILITIES, EQUIPMENT, AND SYSTEMS

The culture facilities include production units, producing-supporting systems, and departments for management and services. The culture facilities (Figure 16.3) are designed for broodstock maintenance and conditioning, as well as rearing of embryos/larvae and microalgae. Seawater supply filtration systems (Figure. 16.4A, B), including pumps (Figure 16.4C), oxygen pumping station (Figure 16.4D), and heating (Figure 16.5) and electrical systems, are the main components of the producing-supporting system (details shown later). Large-scale enterprises commonly build specialized facilities in order to accommodate staff and managers, such as an office building, dining hall, computer room, dormitory building, and so on (Figure 16.6). The most advanced aquaculture industries have laboratories and a research and development unit with essential technical support in water and seed quality monitoring, as well as disease prevention and treatment. Because enterprises are becoming main contributors to technology innovation in China, large-scale research and development departments in the *A. japonicus* industry are cooperating with colleges, universities, and governmental research institutes. They are becoming a leading force of technological innovation in the aquaculture industry.

16.2.1.1 Water supply system

The common structure of the seawater supply is shown in Figure 16.4. The cultures require high water quality with filtration, consisting of sand towers and decantation ponds, sand filter tanks, and UV sterilization stations.

The pump houses consist of a first pump that draws water from the ocean and brings it to the sedimentation tank (Figure 16.4A) and of a second one that supplies the water to the sand filter (Figure 16.4B).

FIGURE 16.3

(A) Typical facilities and (B) tank systems used in *A. japonicus* breeding at the Rushan Branch of Oriental Ocean Co. Ltd.

Photos by Shilin Liu

Different types of pump are used, including submerged pumps (200 m^3 h^{-1}) and pipeline pumps (usually used in the secondary pumping system, with a flow of about 500 m^3 h^{-1}) (Figure 16.4C).

Cast iron pipes (with a SPUA lining to protect the inner wall from seawater corrosion) are used for the seawater intake; other sections of the system utilize polyethylene pipes. There is a drain at the bottom of each nursery tank and algae-culture tank. Wastewater discharges through gutters, which are 50–60 cm below the level of the tank bottom (Figure 16.7).

16.2.1.2 Heat supply system

Because the temperature of ambient seawater in summer is relatively high, it can be used without prior heating. However, seawater temperature is low in spring and autumn and must be heated before it is supplied to the nursery. Moreover, *A. japonicus* is sensitive to fluctuations in seawater temperature, which must be monitored closely to avoid stress. Coal is widely used to heat freshwater to above 80 °C (Figure 16.5A, B). This hot water transfers its energy to culture seawater through heat exchanger plates (Figure 16.5C). Underground well water (geothermal system) can also be used to heat the natural supply of seawater, reducing the use of coal and minimizing environmental pollution. The temperature of

FIGURE 16.4

Typical filtration systems and pumps used for *A. japonicus* breeding at the Rushan Branch of Oriental Ocean Co. Ltd. (A) Sedimentation tank (front) and sand filters (back) for water supply system. A wind generator used to generate electricity in this *A. japonicus* aquaculture facility can also be seen. (B) Sand filter for water supply system. (C) Indoor pump for water supply system. (D) Air pump system.

Photos A, B, and C by Shilin Liu, and D by Yucen Bai

FIGURE 16.5

(A, B) Boiler and (C) heat exchanger for water supply system used for *A. japonicus* breeding at the Rushan Branch of Oriental Ocean Co. Ltd.

Photos A and B by Yucen Bai, and C by Shilin Liu

underground well water is generally up to 14 °C. Plate exchangers can be used to transfer this heat to increase the temperature of ambient seawater when necessary. But this system cannot bring the temperature to the desired values of 15–17 °C required for the *A. japonicus* nursery; hence, a steam boiler is usually used to increase the temperature to the final required level. In addition, each nursery features air conditioning to keep the indoor temperature at a constant level.

16.2.1.3 Oxygen supply system

Most facilities adopt roots blower to supply air to nurseries through pipes. The air flows from main PVC pipes to each tank via flexible plastic hoses, which are fitted with air stones. Typically, eight air stones are placed at the bottom of a 20 m^3 tank; the stones are always secured in place with weights.

FIGURE 16.6

Special buildings to accommodate staff and managers at the Rushan Branch of Shandong Oriental Ocean Co. Ltd aquaculture facilities.

Photos by Shilin Liu

The functions of aeration are as follows:

- Maintain suitable oxygen levels of 4–6 mg L^{-1} in seawater.
- Promote oxidative decomposition of organic matter and nitrification of ammonium nitrogen.
- Disperse larvae and algae evenly, preventing any high-density aggregations due to phototaxis; an even distribution facilitates growth and development.
- Restrain the production of toxic substances, for example the development of bacterial blooms and the multiplication of protozoa.

16.2.1.4 Electric system

The electric support system is an important infrastructure for the nursery and other facilities and equipment. The power distribution room forms the core of this system. The foremost electrical demand for the nursery comes from the pumps, boiler, blowers, and heaters.

16.2.2 TECHNIQUES AND MANAGEMENT

Pioneering work on the hatchery and culture of *A. japonicus* was led by the Japanese. Inaba (1937) first attempted artificial fertilization in *A. japonicus* and Imai et al. (1950) reported its successful artificial rearing. Ishida (1979) noted that thermal stimulation gave good results to induce spawning and Yanagibashi et al. (1984) developed rearing procedures for newly settled juveniles. Xiao and Gu (1981), Li (1983), and Sui et al. (1986) reported on the breeding and culture of *A. japonicus* in China. Since then, the industrial culture of *A. japonicus* has developed in several Asian countries but most significantly in China (James, 1994; Sui, 2004, 2005; Mercier and Hamel, 2013).

FIGURE 16.7

(A) Floor drainage for the nursery tanks used for *A. japonicus* breeding at the Rushan Branch of Oriental Ocean Co. Ltd. (B) Wood planks used to cover the drain.

Photos by Yucen Bai

16.2.2.1 Algae culture

The main species of algae used as food in a nursery of *A. japonicus* are *Closterium* (diatoms), *Chaetoceros*, *Chlorella*, *Isochrysis galbana*, and *Pyramimonas*. The culture can be divided into three phases: maintenance of stock cultures, amplification, and large-scale culture (Zhang et al., 2004). Commonly, there is an algal room in *A. japonicus* facilities, comprised of a light incubator and small burets to preserve algal stock cultures. A clean bench is also needed for inoculation.

The amplification stage is performed in an independent room in which many shelves are available (Figure 16.8). Jars of 5000 mL are filled with liquid nutrient specifically designed for algae. The jars are covered with disinfected newspapers and shaken to aerate the new culture.

The various materials used for large-scale culture consist of white plastic drums of 500–1000 L (Figure 16.9A) and concrete tanks (Figure 16.9B). The ratio of water between the tanks and drums is roughly 5:1. The tanks are always 10 m^3 and have an effective depth of 1 m. Algae rooms should possess good insulation and lighting condition to keep them above 25 °C and 3000 lux, respectively. It is essential to stir the algae cultures manually. Some nutrients are required for the algae to grow and multiply including N, P, Fe, Si as well as vitamins, among others. Formulations and dosage are species-specific.

FIGURE 16.8

Concentrate culture of various algae species in the amplification room of the Rushan Branch of Oriental Ocean Co. Ltd.

Photo by Yucen Bai

FIGURE 16.9

(A) Plastic drums and (B) concrete tanks used for large-scale cultivation of algae of the Rushan Branch of Oriental Ocean Co. Ltd.

Photos by Yucen Bai

Management of an algal culture in China is as follows:

- Direct sunlight should be avoided and fluorescents or halogen lamps should be utilized on overcast days.
- Because bleach solution (10%) is used to disinfect the material (usually adding saturated bleach solution at a concentration of 0.3 L m^{-3} for more than 8 h), the pH must be neutralized to 7.5–8.5 by using sodium thiosulfate before transferring algae into the large-scale culture ponds.

Instruments used should be cleaned to prevent the introduction of copepods and harmful bacteria in the water used for the algae culture. Evaluating the concentration of algae per mL is typically determined using a hemacytometer. Usually, the density of algae in the culture pond is around 200–300 × 10^6 cells mL^{-1}. The algae culture will be pumped into *A. japonicus* larval cultures through specialized pipes. Also, the density of algae in the *A. japonicus* culture should be maintained between 10,000 and 80,000 cells mL^{-1} (with increments of 10,000 cells mL^{-1} daily until the doliolaria stage is reached).

16.2.2.2 Broodstock and larval culture

The breeding of *A. japonicus* can be divided into five stages: conditioning of broodstock; egg collection (spawning), fertilization, and hatching; larval rearing; juvenile rearing; and wintering.

The tank used for the broodstock is usually 4 m^2 (2 m × 2 m) large and 1.2 m deep (Figure 16.10). Broodstock is collected from the wild, sea-ranching areas or ponds. Broodstock should be maintained at a density of 20–30 ind. m^{-3} and a temperature between 13 and 16 °C until it is time to enhance the ripening of the gonads. Broodstock can be fed nutrient-rich diets to accelerate gonad development (see Chapters 7 and 10). Because *A. japonicus* feeds well at 13–16 °C, they should be maintained at this temperature until 10–20 days before spawning induction (however, this method may vary; see Chapter 7). Temperature should then be increased slowly to 17–19 °C until the anticipated spawning induction period. Generally, when the accumulated temperature reaches 800 °C/day, the gonad of *A. japonicus* will be mature and individuals will spawn naturally (Wei, 2008) (see Chapter 7 for more details on spawning induction).

FIGURE 16.10

Concrete tanks used for the broodstock conditioning in the Dajijia Branch of Oriental Ocean Co. Ltd.

Photo by Shilin Liu

During the broodstock conditioning process, natural diets or artificial feed can be used in proportions of 3–5% of *A. japonicus* wet weight per day. Typical diets for *A. japonicus* include kelp powder mixed with marine mud at a weight proportion of 1:8–1:16. The amount of food added every day is dictated by broodstock size and biomass. Maintaining a dark environment is important during this period. Additionally, feeding should be stopped 3–5 days before the expected spawning induction day. As those tanks are generally not under flow through, half the seawater should be changed once a day and continuous aeration with air bubbles should be provided. If mortalities occur, carcasses must be removed promptly and a penicillin treatment might be considered (see Chapter 10 for details on feeding).

Naturally or artificially triggered spawning may be used to obtain competent (fertilizable) *A. japonicus* oocytes for hatchery production (see Chapters 7 and 8 for details). Naturally released gametes are preferred when broodstock is harvested during the normal breeding period or when broodstock conditioning has yielded individuals with fully ripe gonads. This method is obviously the simplest and easiest and provides the highest quality of oocytes. Since *A. japonicus* always spawns at nightfall or during the night, it is difficult to control the amount of gametes released (Wang et al., 2004); it might happen that a very large amount of gametes (especially male gametes) will ruin water quality so that fertilized oocytes (most often polyspermic) do not survive and will need to be discarded. To minimize this possibility, technicians should monitor the broodstock continuously during the potential spawning nights and remove spawners from the pond when they have collected enough spermatozoa and oocytes, thus maintaining a good ratio between gametes to ensure optimal fertilization and survival rates.

Artificial methods are also used to induce *A. japonicus* to spawn. These include temperature shock, UV seawater immersion method, desiccation, and running water stimulation. For example, when the gonad index and the oocyte diameter are more than 20% and 140 μm, respectively, technicians will collect *A. japonicus* broodstock and transfer them in a tank under low light conditions and spray them with seawater using a small plastic pipe. They will thereafter be transferred to a tank filled with clean seawater at 20–21 °C (i.e., thermal shock) (Ito and Kitamura, 1998). Chapter 7 provides more details on the various spawning induction methods.

There are two common fertilization techniques: natural fertilization following spawning (occurring when male and female gametes mix naturally in a pond) and controlled fertilization in a tank or pond (at which time the technician will control the amount of spermatozoa for fertilization). The best temperature for fertilization is around 20–21 °C and the desired spermatozoa density between 3 and 5 per oocyte. The water in the fertilization enclosure should be stirred to prevent fertilized oocytes from agglomerating together and sinking to the bottom. Broodstock should be immediately removed and fertilized oocytes should be transferred into the hatching tank/pond as quickly as possible after counting the number of oocytes.

The density for initial incubation of the early embryonic stages is generally 10 eggs/embryos mL^{-1} and the optimal temperature adjusted between 20 and 24 °C. The pond water should be stirred every 30–60 min and aerated slightly. The essential elements at this stage include controlling density and temperature based on developmental kinetics (see also Chapter 8). When all the embryos have hatched from the fertilization envelope, the ones found in the middle and upper layers of the water column have the highest chance of developing normally. They should be selected for transfer to nursery tank/ponds. Ideally, larvae are transferred immediately after hatching to prevent negative impacts of overly high densities on growth and survival.

At 26–40 h postfertilization, the earliest auricularia larvae are detected in the culture (see Chapter 8). Temperature at this stage should be adjusted to 18–22 °C and the density maintained between 0.3 and

1.5 larva mL^{-1}. The preferred diet is composed of single-celled algae (*Chaetoceros muelleri*, *Nitzschia closterium*, and *Phaeodactylum tricornutum*) or alternatively of yeast *Rhodotorula marina*.

After transfer of the healthiest larvae into larger tanks (5 m long, 2.5 m wide, and 1.8 m deep), 1/3 of the water should be pumped out. Subsequently, new water is added daily during the first 2–3 days until the tank is full. Thereafter, during the early nursery stage, 1/3 of the water will be changed once and then 1/2 of the water will be changed daily for the remainder of the culture. A siphon and filter drum (with silk mesh with a diameter of 0.125 mm) is used to change water; drums are shaken continuously to avoid crushing larvae against the mesh. Except during water change and feeding, ponds/tanks should be aerated and stirred every day. Monitoring water quality several times daily is imperative during this phase.

Ideally, samples of larvae should be examined to establish appropriate feeding frequency and algal density requirement. When the larvae's stomach is adequately filled and the amount of algae in seawater has reached the density of 60,000 cells mL^{-1}, the food supply is considered adequate. If the color of larvae's stomach is pale and algae in seawater are difficult to find under a microscope, then food supply should be increased.

The supply of microalgae to hatcheries is often insufficient to meet the requirements of several simultaneous batches of larvae of *A. japonicus*. The time of first feeding is an important step for successful culture. To avoid mortality and obtain adequate growth and development, *A. japonicus* larvae in commercial culture should be fed within 48 h of the opening of the digestive tract (Sun and Li, 2012). To achieve high growth rates and fast development of auriculariae and maximize their survival in hatcheries, starvation periods ≥6 days should be avoided (Sun and Li, 2014) (see also Chapter 8).

Regular monitoring of larval development is essential for a successful culture. Observing and monitoring the shape of the stomach, the development of the body cavity, identifying the appearance of any deformity, and the development of the lipid spheres are essential components of daily management (refer to Chapter 8 for details on developmental stages and their kinetics).

16.2.2.3 Larval settlement and juvenile rearing

Substrata (settlement plates) should be provided when larvae develop into advanced auriculariae and when doliolariae appear. Clean plastic mesh baskets containing square corrugated boards are commonly used (Liu et al., 2008). When the pentactula larvae have developed their first tube foot (ambulacral podium), they are in the process of metamorphosing from a pelagic to a benthic life style and from a larval to juvenile form. At this time they measure about 300–500 µm in length. Juvenile *A. japonicus* are reared in flow-through systems at a rate that ensures two complete water changes every day. Their diet is mainly composed of benthic diatoms (*Cylindrotheca fusiformis*), marine mud (collected by dredge), and artificial feed made of powdered kelp, scallop meat, compound vitamins, and minerals (see Chapters 8 and 10).

The appropriate water temperature is 24–27 °C before the early juveniles grow into 5–6 mm long individuals. Then the temperature is gradually reduced to 19–23 °C when they reach 2 cm and lowered again to 10–15 °C for juveniles longer than 5 cm. Light intensity below 300 lux is suitable during the daytime with ambient photoperiod. The dissolved oxygen must be above 4 mg L^{-1} and salinity should be maintained above 25 for juvenile *A. japonicus* of all sizes.

16.2.2.4 Wintering

Spawning usually occurs in spring, but it is not before autumn that the first seedling will be available to be dispatched to farms for grow out (some will even be sold the following spring, a year after spawning).

So part of these *A. japonicus* will need to undergo a wintering period at the nursery. They will be kept in the original tanks used to grow the larvae (shown earlier). During that period, about 1/3–1/2 of the water should be changed daily and *A. japonicus* should be transferred to another tank once per week. It is necessary to aerate and maintain the temperature between 6 and 10 °C during that period. The light condition is the same as that of larvae culture. The main feed includes powdered scallop meat and kelp powder (shown earlier and also Chapter 10). The food addition corresponds to about 1.5–4% of the wet weight of *A. japonicus*. The daily addition of food should be divided into 40% of the total amount in the morning and 60% in the evening. Improper or excess feeding and sharp changes in water temperature might induce evisceration in *A. japonicus*. The tanks must be kept clean of viscera and moribund *A. japonicus*. Young *A. japonicus* exhibiting severe ulceration should also be removed to avoid the spread of potential diseases (see Chapter 15 for details on immunology and diseases).

Instead of selling juveniles to grow-out farms, some facilities choose to grow part of their seedlings to commercial size in indoor cement tanks. This is the so-called factory culture system. *A. japonicus* cultured in indoor facilities grow more rapidly than those cultured in larger outdoor ponds. The techniques used are the same as for early juvenile rearing. The temperature should be maintained between 10 and 17 °C, with optimal results at 10–11 °C. However, a complete water change on a daily basis is essential. Animals can be fed twice daily, in the morning and evening, using prepared feed in an amount representing between 1 and 10% of their wet weight. It is important to remove feces and residual unconsumed food. Prepared feed generally includes seaweed and artificial *A. japonicus* meal (containing dried bivalve, kelp powder, vitamins, and minerals) (see also Chapter 10). Quarantine measures during all phases must be used to prevent the spread of viruses, bacteria, and parasites, using preventive measures combined with control measures as needed. Temperature adjustments, water quality treatments, and increase in flow (or water changes) can be implemented to maintain a healthy rearing environment. Continuous monitoring helps detect diseases and mortalities in time; analyses and diagnostics can assist in determining the appropriate course of action or treatment, if required.

16.2.3 CULTURE OF RED VARIANT AND HEAT-RESISTANT *A. japonicus*

In Japan, *A. japonicus* can be subdivided into three variants according to their body color, namely red, green, and black (Choe and Ohshima, 1961) (see also Chapters 3 and 22). The red variant was introduced to China from Japan for large-scale seedling production in 2006 because of its high commercial value. However, comprehensive evaluation of the nutritional qualities of the green and red color variants showed that they were essentially the same (Jiang et al., 2013). Moreover, larval development and growth rate were also almost identical between the red and green variants. In contrast, the larval survival rate for the green variant was >90% to the auricularia stage (about 10 days old), while it was much lower (<30%) in the red variant. It was thus demonstrated that the green variant of *A. japonicus* was easier to rear in captivity than the red one (Soliman et al., 2012). A temperature range between 21 and 24 °C and a salinity of 30 are considered optimal for early development of the red *A. japonicus* (Li et al., 2011). In nature, the red *A. japonicus* lives in a more stable environment than the green one (Nishimura, 1995). However, when the red *A. japonicus* was first introduced to China, it was cultured in shallow ponds where water temperature fluctuated greatly (Jiang et al., 2013), which jeopardized its survival.

In general, *A. japonicus* cannot tolerate high temperatures and most of the adults enter a state of aestivation when temperatures rise above 25 °C (Sui and Liao, 1988; Li et al., 1996; Yang et al., 2006).

Most adult *A. japonicus* will aestivate during summer (see Chapter 11 for details on aestivation). *A. japonicus* enters aestivation in middle to late June on the southern coast of Shandong, in early to middle July on the northern coast of Shandong, and in middle to late August along the Liaodong Peninsula (Dalian area). However, aestivation in different areas ends in the same period, generally from late October to early November, with aestivation lasting between 3–4 months (Li et al., 1996). The sensitivity of *A. japonicus* to high temperature limits its distribution and constrains aquaculture development. Therefore, it is important to improve thermo-tolerance to increase the geographical areas in which this species can be cultured along the coast of China. After heat-shock selection during the larval stages, surviving larvae that metamorphosed into juveniles showed higher thermo-tolerance than individuals not exposed to heat-shock. Therefore, heat shock during early pelagic development might be a useful way to select thermo-tolerant strains of *A. japonicus* (Wang et al., 2011) and expand the areas where *A. japonicus* can be cultivated. However, the potential expansion of the species' geographic distribution into non-native areas should be taken under consideration when assessing such initiatives.

16.2.4 THE APPLICATION OF NEW ENERGY SOURCES IN INDUSTRIAL CULTURE

High energetic demands constitute a large proportion of the total cost of *A. japonicus* aquaculture. Especially for large-scale enterprises, which may spend about 30,000–50,000 USD every month. The heating system is essential and coal is the usual fuel in both North and South China (except Hainan Province). Not only is the annual cost of coal significant, this form of energy is also a source of air pollution. In the context of increasing coal prices and government incentives for energy conservation, using new alternative energy sources and adopting measures to conserve energy are imperative.

Many coastal areas (provinces south of Shandong) receive abundant solar radiation so that large-scale enterprises owning adequate space and funds could utilize solar energy as a source for their heating and electrical needs. One method uses vacuum solar tube collectors to heat the seawater for the cultures (Zhao et al., 2011). The solar energy panels available on the market are designed for heating freshwater; therefore, their use in seawater systems requires some technical modifications and investments. The cooperation between aquaculture and photovoltaic enterprises might lead to a more general usage of solar energy in coastal areas around China in the near future.

Wind energy can be used in aquaculture facilities for transport of seawater and to generate electricity. Rural areas of northwestern China already use wind-driven water transport. Using similar wind-driven pumps to carry seawater might be very cost-effective for aquaculture enterprises (Zhao et al., 2011). In recent years, projects to generate electricity by wind power have developed rapidly in Chinese coastal areas. Because of their geographic location along the coasts where wind and sunshine are abundant, electricity generated from solar and wind energy might well become increasingly popular among *A. japonicus* aquaculture industries.

The Anyuan aquaculture company in Penglai (Shandong Province) built a number of new multi-storey aquaculture facilities for *A. japonicus* (Figure 16.11A) in 2011. Unlike single floor structures typical of traditional culture facilities, their buildings have two floors. The lower floor is similar to traditional facilities, but the upper floor is a transparent greenhouse made of steel frame and polygal panels (Figure 16.11B). This construction uses solar energy to significantly enhance culture efficiency and lower the energy costs. This is a good example of a large-scale aquaculture enterprise in recent years that has adopted innovative energy-saving technologies. So far the majority of *A. japonicus* facilities in China have been using plate heat exchangers; some even purchased advanced heat exchange

FIGURE 16.11

The new multistorey aquaculture facility built by Anyuan Aquaculture Company. (A) Outside view (arrow showing greenhouse section). (B) Inside view of the upper transparent greenhouse for algae culture.

Photos by Rentao Kan

equipment from abroad. The utilization of heat exchangers has decreased heat loss significantly and saved energy and costs. Additionally, energy efficiency could be enhanced to another level if a way to recycle seawater was developed (Jie et al., 2012).

16.3 POND (COFFERDAM) CULTURE

This type of culture was initiated in 1985 with a project called "The Study of Sea Cucumber Artificial Cultivation" led by senior engineer Juhai Qiao of the First Institute of Oceanography of the State Oceanic Administration. This research team placed two sizes of *A. japonicus* seedlings (5–6 cm and 1 cm long) into a shrimp farming pond ~20,000 m^2, which had been enhanced with artificial reefs. After two years of culture, they harvested *A. japonicus*; the survival rate was 35%, including animals that reached commercial specification (about 200 g wet weight) (Qiao, 1988). This laid the ground for a key technique of *A. japonicus* culture in ponds that became a reference for *A. japonicus* farming along the Chinese coast. Concurrently, the nationwide shrimp disease that heavily impacted the shrimp industry

spurred a turn toward *A. japonicus* farming. The ponds used for *A. japonicus* culture come in two models; one is made from modified shrimp ponds and the other uses natural tidal water on fenced shoals and shallow water lowland. Because *A. japonicus* are benthic animals, recreating the appropriate ecological conditions in the ponds is key to success (shown earlier; Qiao and Cheng, 2005).

16.3.1 INFRASTRUCTURES AND SYSTEMS FOR POND (COFFERDAM) CULTURE

16.3.1.1 Ponds

A. japonicus ponds are always square or rectangular, between 1.3 and 2.0 ha and 2–3 m deep. They preferably have a dam foundation built with reef and rock or hard sand. The slope ratio is 1:2.5, and the bottom can be covered by cement or stone boards. The water supply and drainage systems are designed and built carefully. Either the tides change water through large gates, or pumps are used for water exchange. The water inlets and outlets are typically covered with an appropriate mesh to prevent the intrusion of potential predators and the escape of *A. japonicus* (Liu et al., 2002).

16.3.1.2 Substratum

Soft substratum is not suitable for *A. japonicus*. If the bottom of the pond is composed of a thick layer of silt and it is impossible to clean it thoroughly, a hard structure can be placed on top of the soft sediment (shown later). The bottom of the ponds should be cleaned completely after 4–5 years of usage during which organic matter has built up beyond the level desirable to maintain water quality for *A. japonicus*.

Artificial reefs are a crucial element used to transform pond bottoms and guarantee the success of grow out (Qiao and Cheng, 2005). This operation can also enhance survival rate and product quality. Artificial reefs not only provide shelters for *A. japonicus* but are also beneficial for the development of benthic diatoms that provide high-quality food to them. *A. japonicus* lives on the benthos, and artificial reefs were found to provide an excellent living environment for this species (Chen, 2003). The material used and the way it is positioned has important effects on culture success. Artificial reef materials should preferably be porous and multileveled in order to provide enough inhabiting space for *A. japonicus*; the weight of the material used should be appropriate for easy transport and deployment (see also Chapter 18).

Old-fashioned artificial reefs lack scientific design and are simply made by stacking stones and tiles. Because of their simple structure, the food they provide is nonoptimal and space utilization rate by the *A. japonicus* is low with a limited yield improvement.

Zhang et al. (2011) designed a multilayer three-dimensional artificial reef structure (Figure 16.12) and solved the problem of space and food supply for *A. japonicus* culture, especially in cofferdams (Figure 16.13). These structures are composed of a concrete base and multiple layers of corrugated boards that are easily colonized by benthic microalgae and macroalgae. Compared with existing systems, this artificial structure is light and durable and makes collection of *A. japonicus* easier. Through the use of this three-dimensional culture system in cofferdams, yields per unit area have increased considerably. For instance, a cofferdam farm of 5400 ha belonging to the East Ocean Co. Ltd. adopted this artificial reef structure and its per unit area yield has improved from 7–8 to 30–42 ind. m^{-2}.

16.3.2 TECHNIQUES AND MANAGEMENT

A. japonicus pond cultivation involves pond disinfection, water preconditioning, introduction of seedlings, and management.

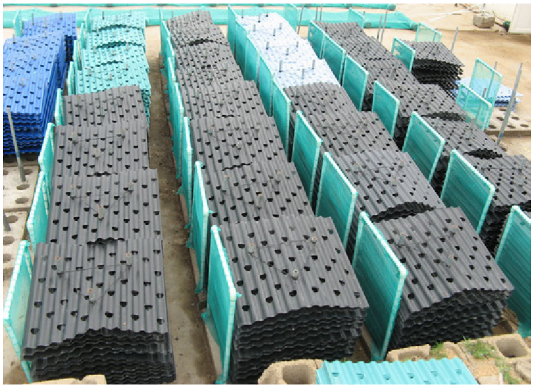

FIGURE 16.12

Multilayer three-dimensional *A. japonicus* artificial reef.

Photo by Libin Zhang

16.3.2.1 Pond disinfection and enrichment

Most *A. japonicus* ponds are repurposed shrimp ponds that are about 2–3 m deep. However, these ponds need to be modified and dredged to a depth of 3–5 m before being used for *A. japonicus*. Once the pond is ready, it can be disinfected for 1–2 days by adding lime powder (about 20–30 g m^{-2}). This procedure begins by filling the pond with seawater to 0.5 m, adding lime powder and draining the pond 1–2 days later. Then the pond is filled with freshwater to absorb excess lime powder and drained again. Subsequently, the pond will be filled again with seawater and organic fertilizer or urea. The total amount of

FIGURE 16.13

Cofferdam (high tide) for the culture of *A. japonicus* in Dalian King Bridge Marine Seedlings Co. Ltd., Liaoning Province.

Photo by Libin Zhang

fertilizer needs to be monitored and more organic fertilizer added as necessary (Zhang, 2012). Beneficial algae will begin to grow and reproduce when the water quality has been stable for 10–15 days. Then the seedlings of *A. japonicus* can be introduced.

16.3.2.2 Introduction of seedlings

There are three main sources of *A. japonicus* seedlings: autumn seedlings, spring seedlings, and natural seedlings. Autumn seedlings refer to those that are harvested from the nursery in September at a typical length of 2–4 cm. Spring seedlings are those that have been cultivated in the previous year and gone through wintering (shown earlier); their length is 5–6 cm. Natural seedlings refer to comparatively larger seedlings that are captured in the wild. Acclimation of seedlings over several hours is recommended prior to their introduction into ponds. This can be done by holding *A. japonicus* seedlings in concrete ponds at an equivalent temperature and salinity. Larger-sized seedlings can be distributed directly on artificial reefs by divers. If the length of seedlings is smaller than 2 cm, they can be placed in bags tied with ropes on the substratum. After they climb out from the open bags by themselves, the empty bags will be removed (Qiao, 1988).

Determining the seedlings' initial density is an important step that will have great impact on culture success. An appropriate density depends on many aspects, including (1) pond size; noting that the aggregating behavior of *A. japonicus* means that it is not necessarily appropriate to introduce proportionally more seedlings in larger ponds; (2) water depth; (3) material, structure, quantity, and design of artificial reefs present in the pond; (4) average size of seedlings; (5) salinity and local average annual temperature of seawater; and (6) quality of food and cultivation skills of the technicians (Qiao and Cheng, 2005).

In general, stocking densities are adapted to the seedlings' initial sizes. Autumn seedlings' stocking density is around 7.5–15 ind. m^{-2}, that of spring seedlings is the highest between 4.6 and 12 ind. m^{-2}, and that of natural seedlings is 3–4.5 ind. m^{-2} (Lin et al., 2003). Generally, a lower stocking density yields greater survival rates under the same conditions. Moreover, the larger the seedlings (with generally greater survival abilities) the more space and more adequate food resources are required.

Experimental trials with *A. japonicus* have been conducted to determine the effect of densities (5, 10, 20, 30, 40, and 50 ind. 100 l^{-1}) on seedlings with an initial body weight of 5.12 ± 0.09 or 6.11 ± 0.26 g that were provided with unlimited food resources. The density of 20 ind. 100 l^{-1} was shown to be the optimum stocking density based on specific growth rates, as well as crude protein and crude lipid contents in tissues. The smaller *A. japonicus* exhibited obvious changes in energetics, such as lower ingestion rate, lower energy devoted to growth but higher respiration, and excretion under stress from the presence of larger individuals (Dong et al., 2010). The availability of food was considered to be the main cause of the uneven growth of the *A. japonicus* seedlings (*A. japonicus* of the same age but various sizes) (Yamana et al., 2008).

16.3.2.3 Management

Similar to other types of aquaculture, management of *A. japonicus* facilities focuses on monitoring and control of water quality, feeding, and prevention and cure of diseases. What is perhaps unique to this culture is that the pond bottom environment must also be monitored and adapted because of the benthic deposit-feeding lifestyle of *A. japonicus*.

Monitoring and control of water quality is one of the most crucial elements of *A. japonicus* aquaculture. It involves careful control of water temperature, salinity, dissolved oxygen level, water

transparency, water color, and pH. Change in the presence of foreign planktic species (e.g., copepods) and their numbers must also be closely monitored. The technical staff should inspect the growth, feeding (food intake), and defecation (fecal pellet output) of *A. japonicus* regularly (Zhang, 2012).

The optimum temperatures for food consumption and for growth were determined to be similar, between 14 and 15 °C. Body size seemed to have a slight effect on the optimal temperature for these parameters. Deduced from daily individual food consumption, the threshold temperature to aestivation (see Chapter 11) for large and medium animals (73.3–139.3 g) was 24.5–25.5 °C, while that for small animals (28.9–40.7 g) was between 25.5 and 30.5 °C (Yang et al., 2005) (see Chapter 8).

Water change is crucial in controlling water quality and it should be adjusted according to the season and particular situation. Flow through is recommended before mid-June. When the water temperature reaches 18 °C, it can be changed at a rate of 20% per day. The water level should be maintained at its highest and water changes should be increased to 50% daily when water temperature reaches 26 °C in mid-August. Water changes are best carried out at night because near-bottom water at that time has low dissolved oxygen levels (Liu et al., 2002).

A. japonicus is adapted to a relatively narrow salinity range and is sensitive to salinity fluctuations. Care must therefore be taken in monitoring this parameter. It is recommended to pump out rainwater from the surface of the ponds to prevent sudden changes in water salinity, especially in summer. It is also essential to add new seawater after rain episodes.

The addition of food in exterior ponds is rarely needed to meet the demands of growing *A. japonicus* in the majority of facilities. Natural food supply, such as microalgae, benthic diatoms, bacteria, protozoa, and organic matter (Zhang et al., 1995), is often enough to sustain their needs. In farms that grow *A. japonicus* in high densities and that recently started up, artificial feed containing fish powder mixed with marine mud has become widely used. The amount of artificial feed used is about 3% of total wet weight of *A. japonicus* once every 2–3 days. However, in the period just after the seedlings are introduced, food addition is no more than once a week because the abundance of already present benthic algae provides adequate food levels (Lin et al., 2003). The annual feeding cycle is as follows: once a week before mid-June; no feeding from mid-June to early October because *A. japonicus* is in a dormant stage (aestivation); once every three days after early October. The amount of feed must be closely monitored and adjusted to avoid decay and deterioration of water quality (see Chapter 10).

Dry kelp and scallop meat powder has been used during *A. japonicus* juvenile rearing in China (see Section 16.1.2.3). Similar mixed diets also showed promising results for the cultivation of subadult *A. japonicus*. However, *A. japonicus* fed powdered kelp alone could not reach the optimum growth rate. According to the specific growth rate (SGR) of tested animals, a formula of 75% powdered scallop meat and 25% powdered kelp is the best diet for the culture of *A. japonicus* (Yuan et al., 2006). Weight gain (WG) and SGR of *A. japonicus* were significantly affected by dietary protein and lipid levels. The diet containing 200 g/kg^{-1} protein (170 g/kg^{-1} digestible protein) with 20 g/kg^{-1} lipid (13 g/kg^{-1} digestible lipid) may be sufficient for the optimum growth of juvenile *A. japonicus* (Seo and Lee, 2011) (see Chapter 10).

16.3.2.4 Disease prevention and cure

This section provides an overview; please refer to Chapter 15 for more details on diseases. Widespread *A. japonicus* diseases emerged in the winter of 2003 and spring of 2004, including "rotting skin," which involves a primary bacterial infection followed by secondary fungal and parasitic infections (Wang et al., 2005). These diseases caused panic in the *A. japonicus* aquaculture industry mainly from the fear that the *A. japonicus* culture would duplicate the shrimp tragedy (that led to mass mortality over years

and abandonment of shrimp cultures in many regions). The "rotting skin" disease is mainly caused by poorly designed/managed culture methods, such as inappropriate management, and decreased resistance to disease favored by the pursuit of increasingly high yields (Chang et al., 2006). The factors that have resulted in the frequent occurrence of *A. japonicus* diseases, and consequent declines in output in recent years, are as follows: (1) excessively high stocking density; (2) *A. japonicus* broodstock from hatchery-bred seedlings; (3) abuse of antibiotics during breeding and cultivation so that seedlings lack immunity and adaptability; (4) improper stocking period and water temperature (<15 °C or >25 °C); (5) deterioration of water quality because of poor monitoring; (6) excessive multiplication of algae, such as *Enteromorpha*, and accumulation of residual unconsumed food items causing deterioration of pond bottom environment; and (7) pollution (oil, eutrophication, and so on). These situations can all elicit evisceration and body wall deterioration in *A. japonicus* (Sun, 2008).

The general solution to most problems lies in scientifically designed culture methods. Specific measures include the following: (1) cleaning of pond bottom regularly, especially for ponds that have been used for several years; (2) using low/conservative stocking densities; (3) introducing seedlings in the appropriate season and at the proper water temperature; (4) avoiding the use of antibiotics as much as possible during the nursery stage; (5) closely monitoring changes in water quality and making prompt adjustments when necessary; and (6) improving the microbial environment of pond substrate through the addition of active bacteria (Qiao and Cheng, 2005).

It has been demonstrated that bacterial infectious diseases, such as "rotting skin" disease, are far less destructive than the white spot disease of shrimp. Controlling the *A. japonicus* disease is not impossible as long as culture techniques are monitored and adjusted on a daily basis.

16.3.3 TRANSPLANTATION OF *A. japonicus* CULTURES IN SOUTHERN LOCATIONS

Southward transfer of *A. japonicus* was first carried out in the early 1980s in the coastal areas of Zhejiang and Fujian provinces, southeastern China (Xiao and Gu, 1981; Sun et al., 2006; Jiang, 2008). In recent years, *A. japonicus* has been successfully transplanted to Guangdong and Guangxi provinces, southern China, and their wet weight shown to have more than doubled within a three-month culture period from November to February (Pang et al., 2010). Further research indicates that culture in winter and spring, optimally from late November to early April, is a viable option for the aquaculture of *A. japonicus* under subtropical conditions (Yu et al., 2012).

16.4 SUSPENDED CULTURE

Suspended culture is commonly used for kelp and scallops, whereas fish cage culture achieved great success in southeastern coastal areas. With the expansion of *A. japonicus* cultures, some scallop and fish farmers have started to culture *A. japonicus* using their original systems and inadvertently developed a new culture mode for *A. japonicus*. Because *A. japonicus* needs a hard substratum to grow, hanging and cage systems should be transformed appropriately. It is also essential for *A. japonicus* to grow in a stable environment, so suspended cultures require certain conditions of current, water quality, and plankton supply. *A. japonicus* cage culture was initially used to hold captured wild *A. japonicus*. In recent years, because of the frequent occurrence of diseases and the decline in product quality associated with pond cultivation, the farming of *A. japonicus* in natural areas (sea ranching) is becoming increasingly popular.

The cost of cage culture (shown later) is less and its harvest rate is higher than typical sea ranching methods, so it is a cost-effective, perhaps more eco-friendly method of cultivating *A. japonicus*.

16.4.1 SYSTEMS FOR SUSPENDED CULTURE

16.4.1.1 Single species cage culture

This system is suited to well-protected inner bays where waves are small, water is clear, nutrient-rich, and frequently turned over. Water depth should be >10 m (Mu and Song, 2005).

Three different cages have been widely used in the suspended culture of *A. japonicus*: (1) cages for *A. japonicus* culture, which are adapted from traditional scallop pens, consist of 10–15 laminar plastic plates with nylon nets covering the plates. In order to facilitate daily management, plastic zippers are used instead of suture for opening/closing the cages. Dense nets of various mesh sizes may also be added in layers to suit the needs of *A. japonicus* of different sizes (Figure 16.14A); (2) Cages adapted from traditional abalone culture consist of six combined separate compartments. A small door is attached on the compartment for opening/closing the cages. The cages are tied to the suspended ropes with buoys floating on the surface of the sea (Figure 16.14B, C); (3) Cages adapted from traditional fish culture consist of chained separate cages with nylon net envelopes. Rope and wood or bamboo are usually used for the frame of the cage. Buoys are tied to the cages for floatation. Dense nets of various mesh sizes are also added in the cages to suit the needs of *A. japonicus* (Figure 16.14D). *A. japonicus* cages can float close to the surface or be placed near the sea floor (Du, 2004). No matter what model is chosen, the cage system must be designed to accommodate the benthic life style of *A. japonicus* (providing substrate for attachment, sheltering, and feeding).

16.4.1.2 Multispecies cage culture

A shallow-water integrated farming device (Figure 16.15) was designed by Yang et al. (2010) by adding a culture plate for *A. japonicus* on the bottom of a scallop pen. This enabled the ecological coculture of scallops and *A. japonicus* in a suspended system. Applying this system in Sanggou Bay (Rongcheng) resulted in maximum growth rates of *A. japonicus* of 0.34%/d^{-1} and the ability of *A. japonicus* to feed on scallop wastes that reached 159.6 kg/ha^{-1} d^{-1} (Yuan et al., 2008) (see Chapter 17 for details on polyculture).

16.4.2 TECHNIQUES AND MANAGEMENT

16.4.2.1 Seedling distribution

The coastal area of Laizhou in Shandong Province is the main scallop-producing area in China and it is also where suspended culture of *A. japonicus* concentrates. Farmers generally place six spring seedlings per layer into the pens in late April, with the number of plates varying between 20 and 50, depending on the conditions and depth of the water. The optimal stocking densities, in terms of net production, were determined to be 22.3 ind. m^{-2} for feed-supplemented pens and 14.1 ind. m^{-2} for non-feed-supplemented pens (Qin et al., 2009).

16.4.2.2 Management

In some conditions, it is necessary to periodically add prepared feed to suspended cultures in a manner similar to pond culture. The feed is usually divided into mesh bags and placed into suspended pens and cages once a week. The feed recipe is the same as the one described for pond cultures and should be distributed following similar seasonal fluctuations (see Section 16.2.2.3).

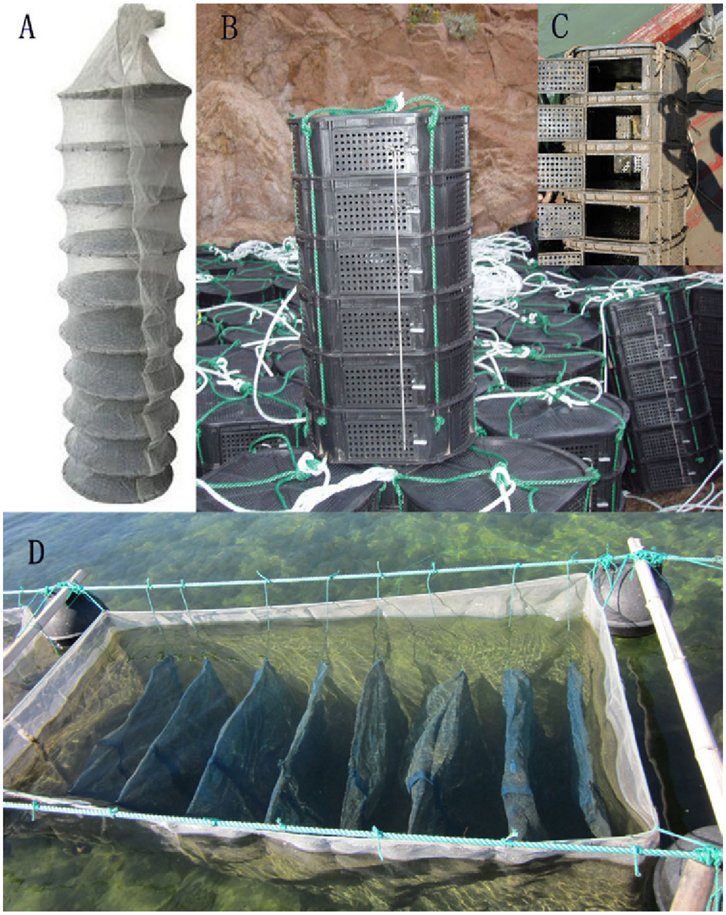

FIGURE 16.14

Different enclosure systems used in suspended culture of *A. japonicus.* (A) The cage adapted from a traditional scallop pen. (B and C) Stack cage systems adapted from traditional abalone culture cages. (D) The cage adapted from traditional fish culture to grow *A. japonicus*.

Photos by Shilin Liu

Apart from weekly feed addition, farmers need to carefully monitor seawater temperature and adjust the depth of the suspended pens or cages at the same time. To prevent damage by waves or strong currents, stones of appropriate size can be placed into the enclosure for ballast and stability (Mu and Song, 2005).

A. japonicus seedlings are mostly bred in indoor artificial conditions. This method has advantages, such as high survival rate under stable conditions during the nursery process. However, as a result of "recycling" the broodstock too many times, using filial generations of hatchery seedlings, or long-term medication, the immune system of seedlings is weakened and they are not necessarily suited to the natural marine environment. This paradox may lead to low quality and poor survival rates following

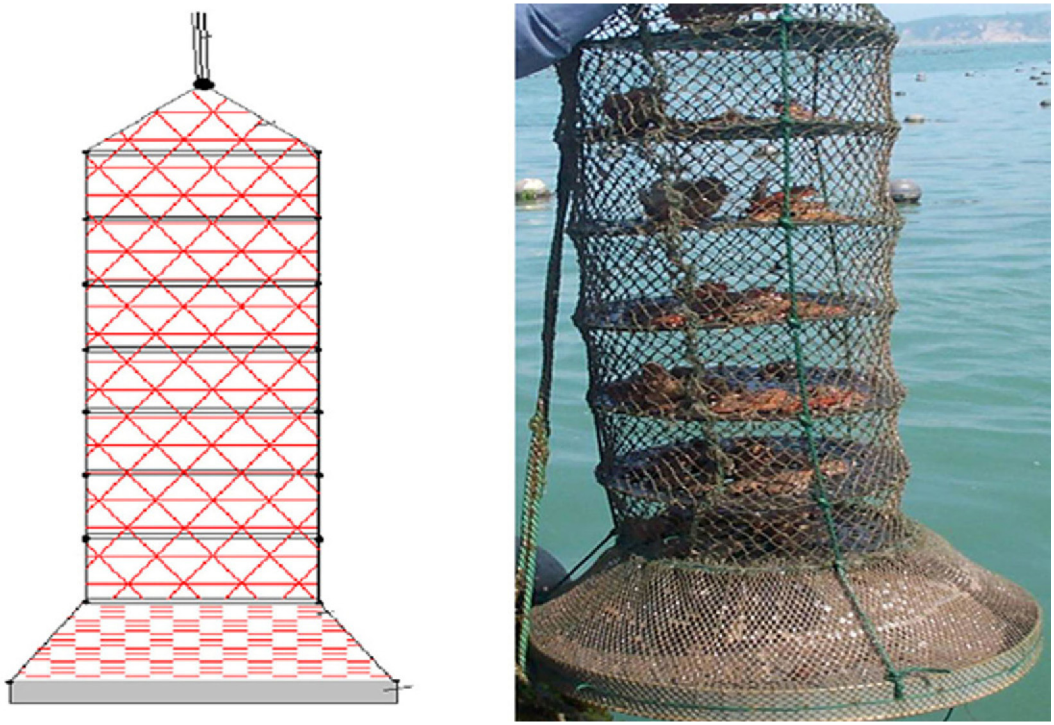

FIGURE 16.15

Multilevel device used for coculture of *A. japonicus* and scallop in shallow water.

Drawing by Ying Liu and photo by Hongsheng Yang

transfer to drastically different outdoor environments. It was recently advocated that the culture of *A. japonicus* seedlings in cages at sea during the juvenile rearing period would improve their endurance and immunity (Ma, 2006). This novel approach should receive more attention in the future.

Transplanting cages to southern (warmer) locations might improve growth rates of *A. japonicus*, allowing them to reach commercial size within a single year. According to recent research, the main limiting factor appeared to be that temperature became too high between April and October (Xing et al., 2012). So the juveniles are usually transplanted to southern locations (e.g., Fujian, Guangdong) in late October to November and the grown-out *A. japonicus* are transported to markets for sale in late March to early April.

16.5 SEA RANCHING

Sea ranching of *A. japonicus* was initiated by the Yellow Sea Fisheries Research Institute in 1980. The results revealed that it was important to add substrata, such as stone blocks, in the *A. japonicus* habitat (Chen, 2003) to enhance their survival and growth.

Owing to the decline of the shrimp aquaculture industry in northern China, most coastal pond facilities have turned to *A. japonicus* farming. In recent years, abuse of antibiotics has developed because of coastal pollution and culture pollution itself, which led to a deterioration in coastal water quality, as farmers were concurrently pursuing higher yield rates. For the sustainable development of this industry, many enterprises started to use stones and make artificial reefs in epicontinental seas, where water quality is high, and to develop *A. japonicus* ranching in areas not favorable for *A. japonicus* originally (Zou and Ma, 2006). Although they are growing slower than through other methods previously described, *A. japonicus* accumulates more bioactive nutrients, which is good for human health (e.g., mucopolysaccharide, fucoidan, saponin, and glycosphingolipid) (Song et al., 2006) and do not exhibit any substantial levels of drugs or other toxic substances in their tissues. Therefore, their quality is obviously higher than *A. japonicus* cultured in artificial ponds or in suspended cages. Because *A. japonicus* is popular as a highly nutritional seafood, products from natural marine areas find greater success in the marketplace than those from ponds and possess higher value-added potential. As coastal pond culture is reaching maximum capacity, sea ranching is becoming increasingly popular and possibly constitutes the future of *A. japonicus* aquaculture.

The conditions necessary to undertake the culture of *A. japonicus* in shallow coastal areas include finding appropriate coastal areas, deploying artificial reefs (if required), and having access to facilities for handling seedlings and harvested products, to ships and boats used in daily management and to nearby dock facilities along the coast.

16.5.1 COASTAL AREAS

A. japonicus thrives in areas where currents are slow and unobstructed, where water is clean, where seaweed grows well, and where the sea floor is composed of rocks or mixed sand and gravel (Sun and Chen, 2006) (see Chapter 5 for details on spatial distribution). Similar conditions are therefore required for successful sea ranching. A survey of water quality, hydrological conditions, sea floor types, and the distribution of other marine organisms present will help determine the appropriate location and period of seeding.

16.5.2 SUBSTRATUM

In both pond culture and sea ranching, artificial reefs can provide shelter and substrate for food resources to grow for the benefit of *A. japonicus* when conditions of a given habitat are not naturally suitable for *A. japonicus*. Porous and multilevel artificial reefs can transform the habitat, increase the available surface area, make the culture space three-dimensional, and improve the production output of *A. japonicus* (Qiao and Cheng, 2005).

16.5.2.1 Natural rocks

The simplest and most widely used materials to build artificial reefs are large natural rocks or stones directly introduced into the coastal area (Figure 16.16). These rocks pile up on the bottom and provide hard substrata on which algae can grow, which in turn form an environment suitable for *A. japonicus*. Although this culture method can achieve great success (Sun and Chen, 2006), the rocks are placed randomly (cast from the surface using boats), which can make dive harvest more complicated. Several enterprises have started to use artificial concrete reefs instead of natural stones as substrata for

FIGURE 16.16

Deployment of boulders into the coastal area to create rocky habitats (reefs) where existing substrate is not suitable for *A. japonicus*.

Photo by Rentao Kan, Shandong Blue Ocean Science & Technology Co., Ltd.

sea ranching. The United States and Japan have long been using artificial reefs to build ocean farms and they have accumulated experience and technologies (Cheng and Hillier, 2011; Seaman, 2007). As *A. japonicus* culture in coastal areas expands in China, artificial reefs are being designed to fit the local needs. For instance, the multilayer modular *A. japonicus* reef and the oyster-shell *A. japonicus* reef have both helped to achieve excellent production yields.

16.5.2.2 Oyster-shell reef

Substrata like rock and seaweed beds supply essential elements for *A. japonicus* to survive, reproduce, and feed. Traditionally, sea ranching was mostly limited to the use of natural rocky areas and relatively stable soft substrate (compacted sand). Bays with fine soft bottoms that lack hard substrata were not amenable to traditional "stone casting" or artificial reefs built with rocks, which tended to sink too deep into the silt.

Yang et al. (2011b) designed the oyster-shell reef (Figure 16.17A) to solve the technical problem posed by bays covered in fine silt that could not *a priori* be used for *A. japonicus* culture. This artificial substrate is composed of polyethylene mesh bags containing 20–75 kg of oyster shells. The weight of this system is light enough to prevent it from sinking into the silt. It can provide food and appropriate anchoring and sheltering areas for *A. japonicus*. Furthermore, natural resources are plentiful to build these artificial reefs. They are cheap to produce and have an extended lifespan of more than 10 years. According to the specifics of given culture areas, a suitable number of artificial structures can be grouped to create an optimum reef environment. The practice will notably increase the primary production of benthic diatoms in the areas and thereby provide adequate space and high-quality natural

FIGURE 16.17

Oyster-shell reef system used for sea ranching of *A. japonicus.* (A) Deployment of the oyster-shell reef to create suitable habitat for *A. japonicus*. (B) Equipment devised to enhance oyster-shell reef-making (see text for description).

Photo A by Tao Zhang, and B by Hui Liu

food for *A. japonicus*. More than 20,000 tons of oyster-shell reef structures have been implanted into previously unsuitable silt-covered coastal areas belonging to Yongsheng Materials Co. Ltd. of Qingdao in Jiaozhou Bay to create a reef area of 667,000 m^2. About 600,000 *A. japonicus* seedlings were initially placed in this artificial reef; the regional density of *A. japonicus* at the end of the experiment was 17.8 ind. m^{-2}, and 11 ind. per reef monomer (one polyethylene mesh bag of oyster shells). It means that each reef monomer had attracted 11 *A. japonicus* on average. Another example is in the soft substratum under suspended cultures in Rongcheng Bay that belongs to the Shandong Lidao Marine Science and Technology Co. Ltd. After introducing oyster-shell reefs equivalent to more than 80,000 tons over 5000 m^2, the *A. japonicus* density was up to 40 ind. m^{-2} after 2,300,000 seedlings were introduced. This demonstrates the enhancement potential of the oyster-shells' structure in a poorly adapted habitat for *A. japonicus* (Zhang et al., 2014).

Yang et al. (2011a) also developed a process to enhance the efficiency of oyster-shell reef-making. The equipment consists of a base, supporting bars, charging canister, and hanging rods (Figure 16.17B). The building process can be divided into three steps: (1) setting the opening of the mesh bag on the hanging rod; (2) pouring oyster shells into the mesh bag using the charging canister; and (3) unloading the mesh bag as it reaches the specified weight and bundling it up. A fivefold increase in efficiency is achieved using this automated process compared to manual crafting. It also makes the reef building process safer by preventing injuries from contact with shells. This process has been widely used in large-scale production of oyster-shell reefs.

16.5.2.3 Multilayered combined reef

Xu et al. (2011) designed a multilayer reef system (Figure 16.18A) that serves three main purposes, which are the development of seaweed beds (Figure 16.18B), shelter for *A. japonicus* (Figure 16.18C), and stock enhancement of commercially important fish (Figure 16.18D). The system is composed of 3–5 layers of cement board and two cement columns. Its ladder layout maximizes exposure to light and can provide adequate food for *A. japonicus* because macroscopic algae are able to attach to each layer. The semihermetic space can provide habitat not only for *A. japonicus* but also for wild commercial fish species, assisting in the stock enhancement process of algae, *A. japonicus*, and wild fish. It has been utilized in an area of 1000 m^2 in the Bay of Haizhou belonging to the Front Three Islands Aquatic

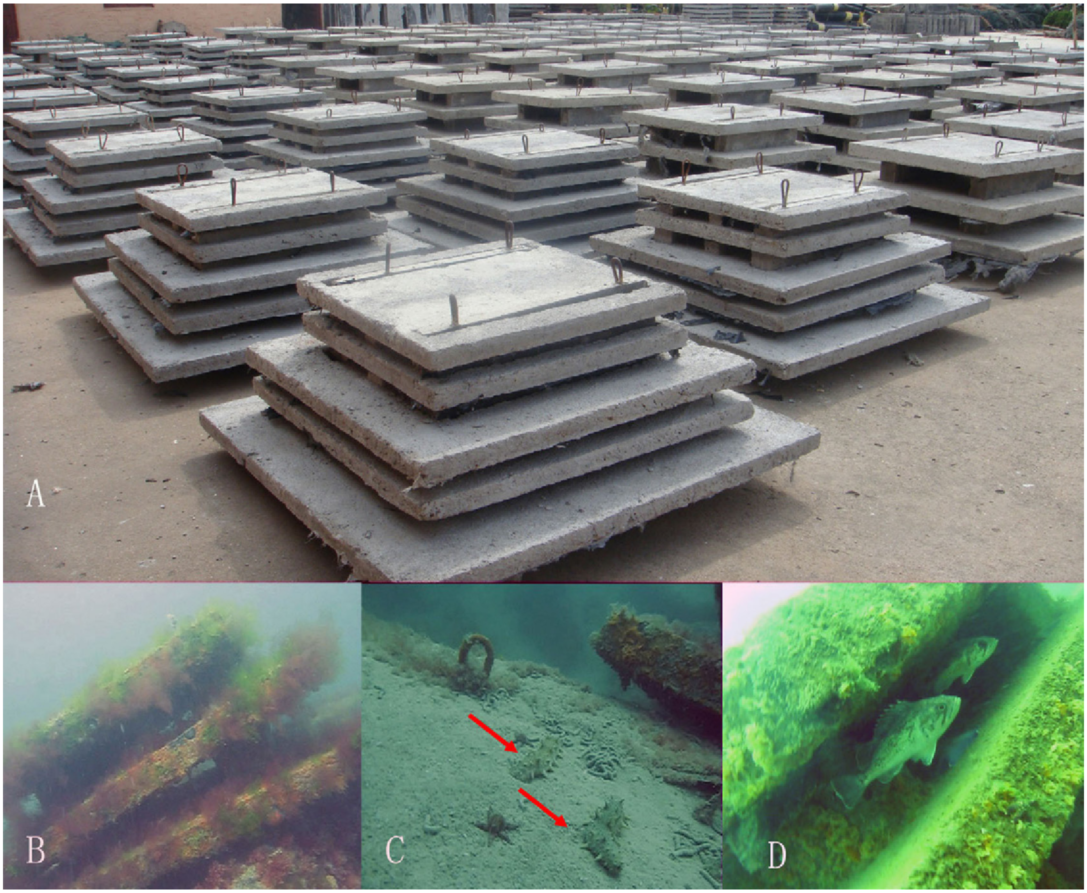

FIGURE 16.18

Multilayered reef system for sea ranching of *A. japonicus* in the Qiansan Islands. (A) Multilayered concrete structure prior to release. (B) Macroalgae growing on the artificial concrete reef. (C) *A. japonicus* visible on the artificial reef (arrows). (D) Fish is also seen in abundance between the concrete layers of the artificial reef.

Photos by Qiang Xu

Development Co. Ltd. The biomass of macroscopic algae attaching to the reef was 111.1–326.4 g m^{-2} and 171.9–276.4 g m^{-2} in spring and summer, respectively. There were on average five *A. japonicus* and six fish (8–20 cm long) per concrete block.

16.5.3 SEEDING AND HARVESTING

Specialized equipment and ships are required to introduce the seedlings into coastal areas. However, harvesting relies on the manual work of divers, so diving equipment is essential.

Similar to the culture of scallops, regular maritime operations are needed to sustain sea ranching of *A. japonicus*. Small ships and docking facilities are required and built on the shore.

The daily management mainly revolves around the elimination of predators and other threats, including the proliferation of the brown seaweed *Scytosiphon* and the presence of predatory sea stars (see Chapter 14 for predation on *A. japonicus*). *Scytosiphon* grows in the intertidal zone and its multiplication and decay can lead to the deterioration of water quality in late spring. At this time, young *A. japonicus* like to hide in seaweed groves to evade light as they enter their aestivation period (see Chapter 11). Hence, proliferation of *Scytosiphon* will lead to the death of numerous *A. japonicus*. As for sea stars, they can ingest whole young *A. japonicus*. Therefore, daily management involves dredging for *Scytosiphon* and removing sea stars and any other potential predators.

16.6 RESTOCKING AND STOCK ENHANCEMENT

The stock enhancement of *A. japonicus* in China began in the 1950s when it mainly operated through the addition of objects like branch bundles in the Swan Lake of Rongcheng, Shandong province to facilitate the metamorphosis and settlement of larvae and serve as shelter for young *A. japonicus* (Ding and Teng, 2005). In recent years, many natural habitats have been destroyed through the deterioration of coastal environments. It is imperative to carry on stock enhancement and restocking of *A. japonicus* populations to restore and even expand its distribution range. Natural coastal areas suited to the reproduction and growth of wild *A. japonicus* should be selected. Substrata can be adapted, healthy seedlings introduced, and the area managed carefully to sustain stable and high-yielding natural *A. japonicus* populations and rich local ecosystems (see also Chapter 18 on habitat enhancement and rehabilitation).

16.6.1 FACILITIES AND SYSTEMS FOR STOCK ENHANCEMENT

The requirement for *A. japonicus* stock enhancement and restocking extend beyond simple sea ranching (Li and Wang, 1994). Although facilities and methods are similar, the particular operations differ because of distinct purposes.

Following the choice of a coastal area with suitable hydrologic and water quality conditions (shown earlier), the seafloor often needs to be adapted to be appropriate for *A. japonicus* growth and multiplication. The specific measures are typically as follows: (1) introducing stones or artificial reef structures (presented earlier) to create an environment where *A. japonicus* can shelter and where seaweed can grow; (2) repurposing existing larger boulders into piles of gravel; the effect is similar to artificial reef systems (Liu et al., 1989); and (3) cultivating macroalgae and plants because *A. japonicus* thrive in clusters or in a large field of them, e.g., *Zostera marina* (Zou et al., 2007).

16.6.2 TECHNIQUES AND MANAGEMENT

The artificial seedlings used in stock enhancement and restocking must be reared carefully to ensure their utmost quality. Seedling harvesting, packing, and transport must be done carefully to prevent injuries. Before introducing the *A. japonicus* seedlings into the sea, they can be provided with high-quality food, such as *Sargassum*, to enhance their nutritional condition (Chen, 2004). The seedlings are usually distributed in ranching areas by divers.

Young *A. japonicus* is found mainly in shallow waters (see Chapter 5), so conservation work is key to the suitability of stock enhancement zones. Regulations against fishing of young *A. japonicus* must be developed and enforced. The principle of controlling the amount and scale of harvests (Chen, 2003) should be followed. This is essential to the development of natural stocks that have reasonable population structures. Meanwhile, the cultivation of useful algae and animals should be increased to create habitats suitable to *A. japonicus* stock enhancement and to sustain a more complete food web and functional ecosystem.

16.7 CONCLUSION

In recent years, following the rapid development of the *A. japonicus* aquaculture industry, people have started to realize the importance of this resource. Conservation of wild *A. japonicus* populations, stock enhancement, and restocking have been implemented and have now achieved promising results. However, there are many problems and challenges. At present, as a result of overfishing, *A. japonicus* is listed as Endangered in the IUCN Red List of Threatened Species (Hamel and Mercier, 2013). Meanwhile, the potential bottleneck effect as a result of repetitively using *A. japonicus* broodstock from hatchery-bred seedlings needs to be more thoroughly investigated. Similarly, the impact of translocating seedlings to the south and introducing *A. japonicus* into new areas on population genetics should be evaluated.

Moreover, the pursuit of excessively high stocking densities and yields has caused serious marine environmental problems (for example, using non-environmentally friendly materials as substrata for *A. japonicus*) in certain coastal areas as well as frequent occurrences of diseases. The abuse of antibiotics and other drugs in nurseries and breeding ponds has led to end-products that exceeded the acceptable concentrations, setting back a flourishing *A. japonicus* aquaculture industry in 2012. Following the output limitations and environmental drawbacks of *A. japonicus* monoculture, more and more aquaculture industries have realized the importance of sustainable development. The investment of the nation and enterprises in the development and utilization of new technologies and new energies in aquaculture is booming. There is reason to believe that the *A. japonicus* aquaculture industry in China and elsewhere will grow to higher, more sustainable levels as long as farmers, enterprises, governments, and researchers fully cooperate in pursuing this common goal.

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CHAPTER

Apostichopus japonicus: A KEY SPECIES IN INTEGRATED POLYCULTURE SYSTEMS

17

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SUMMARY

Integrated multitrophic aquaculture (IMTA) of deposit-feeding sea cucumbers with other species would be beneficial economically (increased harvests) and environmentally (decreasing farm wastes). On the eastern Asian coast, the sea cucumber *Apostichopus japonicus* is considered an excellent candidate for marine IMTA systems. The use of *A. japonicus* can mitigate the accumulation of particulate organic waste produced by other species, due to its deposit-feeding behavior. The experimental studies and commercial practices of sea cucumber IMTA in China started in the late 1980s, and the feasibility of coculture of *A. japonicus* with fish, shrimp, bivalves and/or macroalgae, abalone, and sea urchin has been tested in China, Japan, and Republic of Korea in recent years. Moreover, the bioremediation capability of *A. japonicus* on organic wastes discharged from bivalves and/or macroalgae systems has been evaluated in the context of coculture or polyculture. Future breakthroughs in the field of sea cucumber IMTA are anticipated in the years to come.

Keywords: *Apostichopus japonicus*; bioremediation; eastern Asia; economic and ecological benefits; integrated multitrophic aquaculture; polyculture; sea cucumber

17.1 INTRODUCTION

The concept of coculture or polyculture has been proposed and developed in recent years in an effort to implement sustainable aquaculture practices. It is deemed desirable to accommodate two or more species in an aquaculture system when they are ecologically compatible, i.e., they require similar environmental conditions, and do not compete for food or space (e.g., Neori et al., 2000; Kang et al., 2003; Zhou et al., 2006; Yuan et al., 2008). Mixing animals that occupy different trophic levels is the basis of environmentally friendly polyculture systems (Neori et al., 2004).

It is speculated that deposit-feeding sea cucumbers may serve as effective scavengers in polyculture systems. The temperate species *Apostichopus japonicus* is widely distributed along the coasts of northern China, southeastern Russia, Japan, Republic of Korea, and Democratic People's Republic of Korea (Liao, 1997). *A. japonicus* is characterized by high ingestion rates of deposited organic materials as well as high

Yuan, X., Zhou, Y., Mao, Y. 2015. Chapter 17. *Apostichopus japonicus*: a key species in integrated polyculture systems. In: Yang, H., Hamel, J.-F., Mercier, A. (Eds.), The Sea Cucumber *Apostichopus japonicus*. History, Biology and Aquaculture, Academic Press, pp. 323–332.
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market value and demand, which make it an excellent candidate in mitigating particulate organic waste produced by other species in mariculture systems. There have been many experimental studies and commercial trials focused on the use of sea cucumbers in integrated multitrophic aquaculture (IMTA) in Asia, mainly in China, Japan, and Republic of Korea. Their aim was generally to test the feasibility of coculturing *A. japonicus* with a variety of other species, including fish (Yu et al., 2012; Yokoyama, 2013a), shrimp (Xu and Zhu, 2002), macroalgae and/or bivalves (Zhang et al., 1990; Yang et al., 2000, 2001; Zhou et al., 2006; Yuan et al., 2008; Yokoyama, 2013b), abalone (Zhang et al., 1993; Chang and Hu, 2000; Kang et al., 2003; Wang et al., 2007b; Fang et al., 2009; Qi et al., 2013), and sea urchin (Wang et al., 2007a, 2008). Another objective is to evaluate the bioremediation potential of *A. japonicus* on organic wastes produced by other aquaculture species. However, less attention is being devoted to this particular aspect of coculture feasibility (Zhou et al., 2006; Yuan et al., 2008, 2012, 2013). Clearly, this is an area where more research is needed for developing optimal coculture systems with the sea cucumber *A. japonicus*.

17.2 TYPES OF SEA CUCUMBER IMTA IN EASTERN ASIA

17.2.1 SEA CUCUMBER AND FISH

Organic wastes from fish farming (feces and unconsumed feed) can be adequate food sources for cultivating deposit-feeding sea cucumbers; this becomes the ecological rationale for coculturing them. Yu et al. (2012) reported on the feasibility of cage-culturing *A. japonicus* underneath cultures of various species of fish, such as *Lutjanus erythopterus*, *Epinephelus fario*, and *Rachycentron canadum* in the Bay of Dapeng, southern China. The observed survival rate and specific growth rate (key indices used to evaluate the success of coculture practices) of *A. japonicus* reached 100% and 1.05% d^{-1}, respectively, during winter; however, those data decreased sharply to 65% and 0.44% d^{-1} in spring. Moreover, all sea cucumbers died during summer due to the high water temperature in the bay (located outside the natural distribution range of *A. japonicus*). Unfortunately, there has been no coculture of *A. japonicus* to date with fish in Liaoning and Shandong provinces (northern China), which are the most suitable areas to conduct sea cucumber IMTA with fish. Meanwhile, Yokoyama (2013a) tested the growth of *A. japonicus* in the Bay of Gokasho (central Japan) below fish cages for 238 days and found high survivorship (96%) and specific growth rate (1.9% d^{-1}); all sea cucumbers grew to the market size after another 307 days. He also observed a density-dependent effect on the growth of cocultured sea cucumbers, suggesting that there was competition between sea cucumber individuals for the limited food supply in the context of sea cucumber IMTA with fish.

17.2.2 SEA CUCUMBER AND SHRIMP

Interest in sea cucumber IMTA with shrimp in China has been primarily pursued as a means of removing particulate wastes (uneaten feed and feces) from intensive shrimp farming and preventing shrimp disease outbreak, which has plagued the shrimp aquaculture industry in China since 1992. A commercial IMTA system of shrimp *Penaeus* sp. and *A. japonicus* in shrimp ponds (on a total surface area of 10 ha) was performed in Pulandian Bay, west of the Liaoning Peninsula, in 1998–1999 (Xu and Zhu, 2002). In each 1-ha pond, 150,000 juvenile shrimp (1 cm in body length) and 53,000 juvenile sea cucumbers (5–8 cm in body length) were cocultured and after a period of one year, 459 kg shrimps and 575 kg sea cucumbers (overall market value above 10,000 USD) were harvested. Attention should be

paid to animal size during cohabitation in IMTA since large shrimp could eat small sea cucumbers (Xu and Zhu, 2002). Unfortunately, there has been no further experimental study or commercial initiatives involving the coculture of *A. japonicus* and shrimp since then, despite the apparent ecological and economic benefits of the sea cucumber IMTA with shrimp.

17.2.3 SEA CUCUMBER AND BIVALVES/MACROALGAE

Sea cucumber coculture with bivalves in China was initially investigated in the late 1980s. Zhang et al. (1990) first experimented with the coculture of the scallop *Chlamys farreri* and the sea cucumber *A. japonicus* in lantern nets. There was a 2.9-fold weight increase for juvenile sea cucumbers (initial weight: 24 g) after 11 months and an optimal density of 20 sea cucumbers per net was recommended (Zhang et al., 1990). In an effort to integrate the locally available sea cucumber (*A. japonicus*) in bivalve culture systems along the coasts of China, three types of coculture apparatus were explored, which were modified slightly from the lantern nets largely used in bivalve culture. The newly designed apparatus is cheaper and easier to make (Figures 17.1 and 17.2) (Yuan et al., 2008). Zhou et al. (2006) found that the stocking density of 34 sea cucumbers (20 g in body weight) per net was optimal when cocultured with bivalves. Growth rates of 0.09–0.31 g ind.$^{-1}$ d^{-1} were achieved in Sishili and Jiaozhou bays in northern China. Yuan et al. (2008) concluded that *A. japonicus* could be cocultured with the Zhikong scallop *Chlamys farreri*, the Pacific oyster *Crassostrea gigas*, and the Japanese scallop *Patinopecten yessoensis*. Because a density-specific growth rate was found, a stocking biomass of 200 g of sea cucumber per net was recommended in Sanggou Bay, northern China (Yuan et al., 2008). In Japan, Yokoyama (2013b) performed a field experiment to culture *A. japonicus* in PVC containers below a raft of Pacific oyster *Crassostrea gigas* in Gokasho Bay. After 216 days, *A. japonicus* grew from 0.08 to 5.5 g with 100% survival rate.

Yang et al. (2000, 2001) developed a bivalve–kelp–holothurian IMTA system in the Sishili Bay on the west of Shandong Peninsula (China). They determined optimal stocking densities of 23 ind. m^{-2} for the bivalve (*C. farreri*), 0.71 ind. m^{-2} for kelp (*Laminaria japonica*), and 2 ind. m^{-2} for the sea cucumber (*A. japonicus*) in this polyculture system.

FIGURE 17.1

Lantern nets used for coculturing bivalves with sea cucumbers in suspended culture systems.

Photos by Yuze Mao

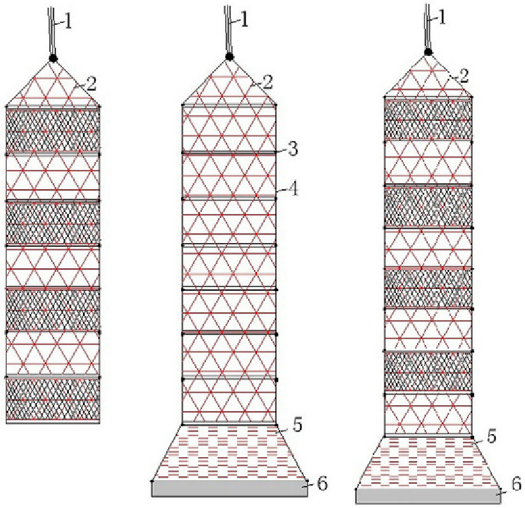

FIGURE 17.2

Schematic drawing of the lantern nets used for coculturing bivalves with sea cucumbers in coastal suspension culture system. (1) sling; (2) lantern nets; (3) board; (4) fixed rope; (5) fine nets; and (6) culture board.

Drawing by Ying Liu

17.2.4 SEA CUCUMBER AND ABALONE OR SEA URCHIN

Macroalgae-foraging species, such as abalone or sea urchin, are considered good candidates for coculture with deposit-feeding sea cucumber, mainly because the abundance of feces discharged from the former could be used as a food source by the latter. Zhang et al. (1993) demonstrated the success of an IMTA system that combined the abalone *Haliotis discus* and the sea cucumber *A. japonicus* along the coast of the Shandong Peninsula. A stocking density of 120 ind. m^{-2} of abalone (1.32 mm in body length) and 5–10 ind. m^{-2} of sea cucumber (17.8 g in body weight) in land-based farming was determined to yield the best results (Zhang et al., 1993). Subsequent field trials of sea cucumber IMTA with abalone were conducted using either artificial reefs or cages (Figure 17.3) in intertidal ponds of Shandong Peninsula; harvests and economic benefits were promising (Chang and Hu, 2000; Li et al., 2001). In Republic of Korea, Kang et al. (2003) cocultured *A. japonicus* (5.0 ± 1.2 g) and the abalone *H. discus* (0.35 ± 0.12 g) in circulating culture tanks under laboratory conditions to reduce seawater pollution from the hatchery during the overwintering period. They observed that the ammonia and nitrite contents decreased and that the survival and growth rates increased in the cocultured group, in contrast to abalone cultured alone in the same experimental setup (Kang et al., 2003). A laboratory-scale experiment suggested a slightly different optimal stocking density of 200 ind. m^{-2} of abalone (8.75 g in body weight) with 5 ind. m^{-2} of sea cucumber (2.24 g in body weight) (Wang et al., 2007b).

Sea cucumber IMTA with abalone are now used at a commercial scale and this practice is common in intertidal ponds (Figure 17.4) and offshore in the Shandong and Liaoning provinces of China. This kind of polyculture produced a growing number of both species under aquaculture facilities over the last 10 years. For example, offshore coculture of *A. japonicus* and *H. discus* produced annual yields that

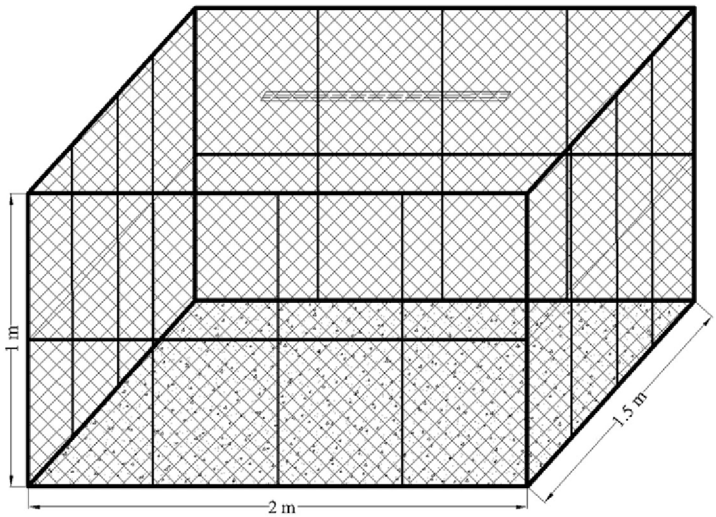

FIGURE 17.3

Schematic drawing of the cage used for the coculture of sea cucumber with abalone in intertidal ponds. A zipper on the top of the cage can be opened for feeding abalone with kelp; the cage is placed on the bottom of ponds.

Drawing by Xiutang Yuan

FIGURE 17.4

Intertidal ponds where the coculture of sea cucumber with abalone occurs in the Shandong and Liaoning provinces of northern China.

Photo by Xiutang Yuan

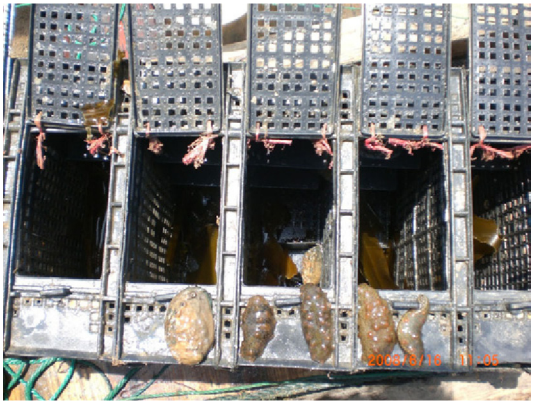

FIGURE 17.5

A cage system used for suspended coculture of sea cucumber with abalone in shallow waters.

Photo by Zhanhui Qi

reached 600 tons of sea cucumbers and 80 tons of abalone in Zhangzidao Island off the east of Liaoning Peninsula (China) (Barrington et al., 2009).

In 2009, a suspended coculture system of *A. japonicus* with *H. discus* as well as *L. japonica* (Figure 17.5) was also developed in the nearshore waters of Lidao Bay, in the eastern region of Shandong Peninsula. The cultivated abalone fed on kelp and sea cucumber fed on abalone feces. In an 8-ha plot, a normal commercial density of 250 juvenile abalones (52 mm in shell length) per cage and serial densities of 3, 6, 12, and 24 juvenile sea cucumbers (65 g in body weight) per cage were cocultivated. The average body weight of cocultured sea cucumbers increased by 96% during the seven-month field trial, with an average specific growth rate of 0.33% d^{-1} (Fang et al., 2009). Furthermore, feasibility of offshore coculture of the abalone *H. discus* and the sea cucumber *A. japonicus* in abalone cages suspended on long lines was investigated in Sanggou Bay, northern China (Qi et al., 2013). All the cocultured animals survived and the annual average specific growth rates for sea cucumbers and abalones were 0.18 and 0.16% d^{-1}, respectively.

Compared to sea cucumber IMTA with abalone, studies of IMTA combining sea cucumber and sea urchin have received much less attention. A laboratory-scale IMTA of the sea cucumber *A. japonicus* with the sea urchin *Strongylocentrotus intermedius* was performed. Wang et al., (2007a) observed that *S. intermedius* cocultured with *A. japonicus* achieved survival rates of 90, 94, and 89% and SGRs of 0.47, 0.41, and 0.18% d^{-1} in sea urchin–sea cucumber individual ratios of 10:5, 20:5, and 30:5, being higher by 29, 34, and 26% in survival rates and 3, 10, and 23% in SGRs than those of the same density sea urchin monoculture during 151 days. Meanwhile, cocultured sea cucumbers survived at 85 and 55%, increased body weights of 1.12 and 0.83 g in the 5:10, 5:20 ratios, but survived at only 26% and decreased body weight of 1.3 g in the 5:30 ratio. Furthermore, Wang et al. (2008), have also tested the survival and growth of animals in sea urchin–sea cucumber individual ratios of 11:3, 11:5, and 11:10. They found all cultivated animals survived in 77 days and sea urchins achieved SGRs of 1.16, 1.14, and 1.17% d^{-1}, which were all significantly higher than that in sea urchin monoculture (1.06% d^{-1}); sea cucumbers gained SGRs of 0.22, 0.19, and 0.12% d^{-1}, respectively, in the mentioned density

ratios. According to these results, they recommended an optimal stocking density of 11 juvenile sea urchins (1.4 g in body weight) and three juvenile sea cucumbers (3.4 g in body weight) for further field practice.

17.3 BIOREMEDIATION CAPABILITY OF *A. japonicus* WHEN COMBINED WITH BIVALVE AND/OR MACROALGAE

The advantages of IMTAs that combine sea cucumber with other commercial species would be beneficial economically and environmentally. In this context, the bioremediation ability of a deposit-feeding sea cucumber is an important index to assess its environmental benefits. Up to now, many studies have dealt with the feasibility of coculture or polyculture of *A. japonicus* with various species; far fewer, however, have evaluated the bioremediation potential of this species on organic wastes produced by the cocultured species in IMTA systems. Moreover, these evaluations were only limited to the sea cucumber IMTA systems with bivalves and/or macroalgae (see Table 17.1). For example, a laboratory experiment demonstrated that *A. japonicus* could survive well on bivalve biodeposits, with a feeding rate of 1.82 g dry biodeposits ind.$^{-1}$ d^{-1}, showing a great bioremediation potential when cocultured with bivalves (Zhou et al., 2006). Furthermore, a field study performed in Sanggou Bay (northern China) showed that *A. japonicus* cocultured with bivalves in lantern nets could ingest the biodeposits generated by the bivalves at rates of 0.175 g g^{-1} d^{-1} (in summer), 0.099 g g^{-1} d^{-1} (in autumn), and 0.005 g g^{-1} d^{-1} (in winter). Extrapolation of these results indicated that sea cucumbers cocultivated with bivalves in lantern nets would ingest between 4.5 and 159.6 kg dry biodeposits ha^{-1} d^{-1} depending on the season (Yuan et al., 2008).

As just shown, studies have assessed the bioremediation capabilities of *A. japonicus* by measuring feeding rates on organic wastes. Of the ingested organic matters, however, only the portion that is processed and retained can be considered as effective remediation. Therefore, the effective remediation

Table 17.1 Bioremediation Capabilities in the Deposit-Feeding Sea Cucumber *A. japonicus* Cocultured with Bivalves and/or Macroalgae

Body Weight (g)	Waste as Feed	Feeding Rate	Scavenging Efficiency	References
10.6 ± 1.5	Scallop biodeposits	1.82 ± 0.13 g ind.$^{-1}$ d^{-1}	—	Zhou et al., 2006
32.5 ± 1.0	Scallop biodeposits and mixed diets with powdered algae	0.62–2.43 g g^{-1} d^{-1}	—	Yuan et al., 2006
37.7 ± 1.9	Scallop biodeposits	0.005–0.175 g g^{-1} d^{-1}	—	Yuan et al., 2008
32.5 ± 1.0	Scallop biodeposits and mixed diets with powdered algae	35.8–181.2 mg C g^{-1} d^{-1} 6.1–14.3 mg N g^{-1} d^{-1}	0.83–4.57 mg C g^{-1} d^{-1} 0.28–0.76 mg N g^{-1} d^{-1}	Yuan et al., 2012
36.6 ± 1.3	Scallop biodeposits (75%) with powdered algae (25%)	0.0–131.3 mg C g^{-1} d^{-1} 0.0–11.9 mg N g^{-1} d^{-1}	0.0–38.5 mg C g^{-1} d^{-1} 0.0–1.9 mg N g^{-1} d^{-1}	Yuan et al., 2013

potential is not a single parameter (e.g., the amount of waste consumed by sea cucumbers), but a new index through which the overall physio-ecological process should be taken into consideration. Based on this concept, a more precise definition of the bioremediation capability, especially the scavenging efficiency of biogenic elements, such as carbon and nitrogen, is needed. It is theorized that only nutrients devoted to growth can be considered an effective process in bioremediation practice. The unassimilated carbon and nitrogen that passes with feces is not removed from sediment and consequently still categorized as a self-pollutant in the coculture system. Furthermore, part of the assimilated carbon and nitrogen, which was converted to metabolites in the forms of dissolved carbon (such as HCO_3^- and CO_3^{2-}) and inorganic nitrogen wastes (such as NH_4^+), could be absorbed through photosynthesis and growth of macroalgae, such as *L. japonica* or *Gracilaria lemaneiformis* (Mao et al., 2009) in the suspended polyculture area where the bivalves, macroalgae, and sea cucumbers coexist. Without the deposit-feeding sea cucumber, the dissolved carbon and the inorganic nitrogen could not be isolated from the particulate organic wastes. Therefore, the scavenging efficiency of biogenic elements, such as carbon (SE_C) and nitrogen (SE_N), could be estimated as $SE_C = C_C - F_C$ and $SE_N = C_N - F_N$, where, C_C (C_N) is the ingested carbon (nitrogen); F_C (F_N) is the defecated carbon (nitrogen) (Yuan et al., 2012, 2013).

In this context, a laboratory experiment was designed to calculate the scavenging efficiencies of carbon (SE_C) and nitrogen (SE_N) of *A. japonicus* from the perspective of nutrient budgets (Yuan et al., 2012). Five types of organic waste (pure dried biodeposits; 75% dried biodeposits and 25% powdered algae; 50% dried biodeposits and 50% powdered algae; 75% dried biodeposits and 25% powdered algae; pure powdered algae) were used to feed sea cucumbers in order to simulate possible proportions in biodeposits and macroalgae detritus from the suspended coculture systems of bivalves and/or macroalgae. Results showed that *A. japonicus* could effectively ingest such particulate waste at rates ranging between 35.8 and 181.2 mg C g^{-1} d^{-1} and 6.1 and 14.3 mg N g^{-1} d^{-1} for carbon and nitrogen, respectively. Furthermore, ingestion rates increased with increasing carbon and nitrogen contents in the particulate waste. Scavenging efficiencies for carbon and nitrogen (SE_C, SE_N) in the sea cucumbers fed particulate waste were 0.8–4.6 mg C g^{-1} d^{-1} and 0.3–0.8 mg N g^{-1} d^{-1}, respectively. Moreover, a trend was observed of increasing SE_C and SE_N with increasing carbon and nitrogen contents of the tested waste. A positive correlation between SE_C or SE_N and carbon and nitrogen contents (C or N) was also noticed; this relationship is better described as: $SE_C = 0.7368 + 14.9488_C$ or $SE_N = 0.2281 + 0.2202_N$.

To model the scavenging efficiencies in *A. japonicus* under different temperature regimes, Yuan et al. (2013) fed them with a diet containing 75% biodeposits and 25% powdered algae to simulate the organic waste from the bivalve–macroalgae culture system. This study showed that the SE_C and SE_N ranged from 0.0 to 38.5 mg C g^{-1} d^{-1} and 0.0 to 6.7 mg N g^{-1} d^{-1}, and they increased with temperature from 5–15 °C, but decreased when temperatures went above 15 °C. Particularly at 30 °C, the test sea cucumber *A. japonicus* fully entered into aestivation, stopped feeding and reduced its scavenging efficiency to zero, underlining a thermal limitation to bioremediation. In the management of IMTA, priority should be given to juveniles of *A. japonicus* with a weight of 10–30 g since they are not affected by aestivation within a temperature range of 3–28 °C (Liao, 1997) in their geographic distribution. Moreover, when sea cucumbers grow to more than 70 g (minimum aestivating size), attention should be paid to the low scavenging capability at relative high temperatures (>25 °C), especially during aestivation, because sexually mature individuals aestivate for >100 days (Liu et al., 1996; Yuan et al., 2008).

17.4 CONCLUSION AND FUTURE PROSPECTS

Several types of IMTA were developed that combine the sea cucumber *A. japonicus* with fish, shrimp, macroalgae and/or bivalves, abalone and sea urchin to mitigate the accumulation of aquaculture waste in the coastal waters of China, Japan, and Republic of Korea. The feasibility of *A. japonicus* coculture with ecologically compatible species was tested, and the bioremediation capabilities in the sea cucumber IMTA with bivalve and/or macroalgae were assessed. Further study in this field should focus on developing suitable facilities for sea cucumber IMTAs and optimizing techniques to increase economic and environmental benefits.

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CHAPTER

HABITAT ENHANCEMENT AND REHABILITATION

18

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SUMMARY

This chapter outlines the main threats to coastal environments and to populations of *Apostichopus japonicus* and reviews the methods currently being developed to mitigate habitat loss. In the wild, *A. japonicus* usually lives in coastal areas where there are healthy rocky reefs (covered with macrophytes) or in seagrass beds at depths of 3–10 m. Due to pollution of the marine environment and overfishing, the habitat of *A. japonicus* has deteriorated gradually, and suitable environments for its populations to thrive are becoming rarer every year. Efforts are therefore being made to enhance or replenish the wild stocks of *A. japonicus* by deploying appropriate types of artificial reefs, and by rehabilitating seagrass beds.

Keywords: *Apostichopus japonicus*; artificial reef; enhancement; habitat; rehabilitation; sea cucumber; seagrass bed

18.1 INTRODUCTION

The native habitat of *Apostichopus japonicus* comprises coastal zones between 3 and 10 m depth with abundant rocks, macrophytes, or seagrasses (see Chapter 5 for details on distribution and population structures). Coastal areas that are essential for this species are also among China's most important traditional marine aquaculture waters. With the unprecedented development of aquaculture in recent years, coastal environments have been degrading (Yuan et al., 2001; Liu and Diamond, 2005; Meng et al., 2008), increasing the incidence of diseases and mortalities among cultured organisms, compounded by frequent natural disturbances, such as sea-ice, typhoons, red tides, and oil pollution. In order to improve environmental conditions along the coast, habitat rehabilitation and implementation of a "clean production" act have become the primary challenges of marine development. At present, solving environmental problems is mainly focusing on mitigating the effects of overfishing and pollution.

18.1.1 OVERFISHING

Overfishing is a form of overexploitation in which stocks of aquatic organisms are depleted to unsustainable levels. *A. japonicus* offers a good example; it has been exploited throughout its range. In China, wild stocks are considered to be largely extirpated and it is now being massively produced through hatcheries and sea ranching (see Chapter 16) to meet commercial demands (Hamel and Mercier, 2013).

Lin, C., Zhang, L. 2015. Chapter 18. Habitat enhancement and rehabilitation. In: Yang, H., Hamel, J.-F., Mercier, A. (Eds.), The Sea Cucumber *Apostichopus japonicus*. History, Biology and Aquaculture, Academic Press, pp. 333–352. http://dx.doi.org/10.1016/B978-0-12-799953-1.00018-0

Bottom trawling itself is an important threat to the marine environment in China. The small mesh size of fishing nets and the bottom metal chains used for inshore fisheries (mainly to catch fish) in areas where *A. japonicus* naturally occurs, have caused serious damage to benthic habitats and created vast barren areas (Li and Chen, 2009; Dong et al., 2010).

Destructive fishing practices also made the biomass of *A. japonicus* decrease sharply. In Yuehu lagoon (Swan Lake) in Rongcheng, Shandong Province, China, for example, the environment was largely pristine before the 1950s, but from 1957 onward, local residents started exploiting this area. The production of dried *A. japonicus* was between 1000 and 3200 kg annually in the 1960s and 1970s. In order to prevent the escape of juvenile *A. japonicus* from the lagoon, an artificial dam was built in 1979 (Figure 18.1) so that the water could only be exchanged through a narrow gate. Subsequently, the

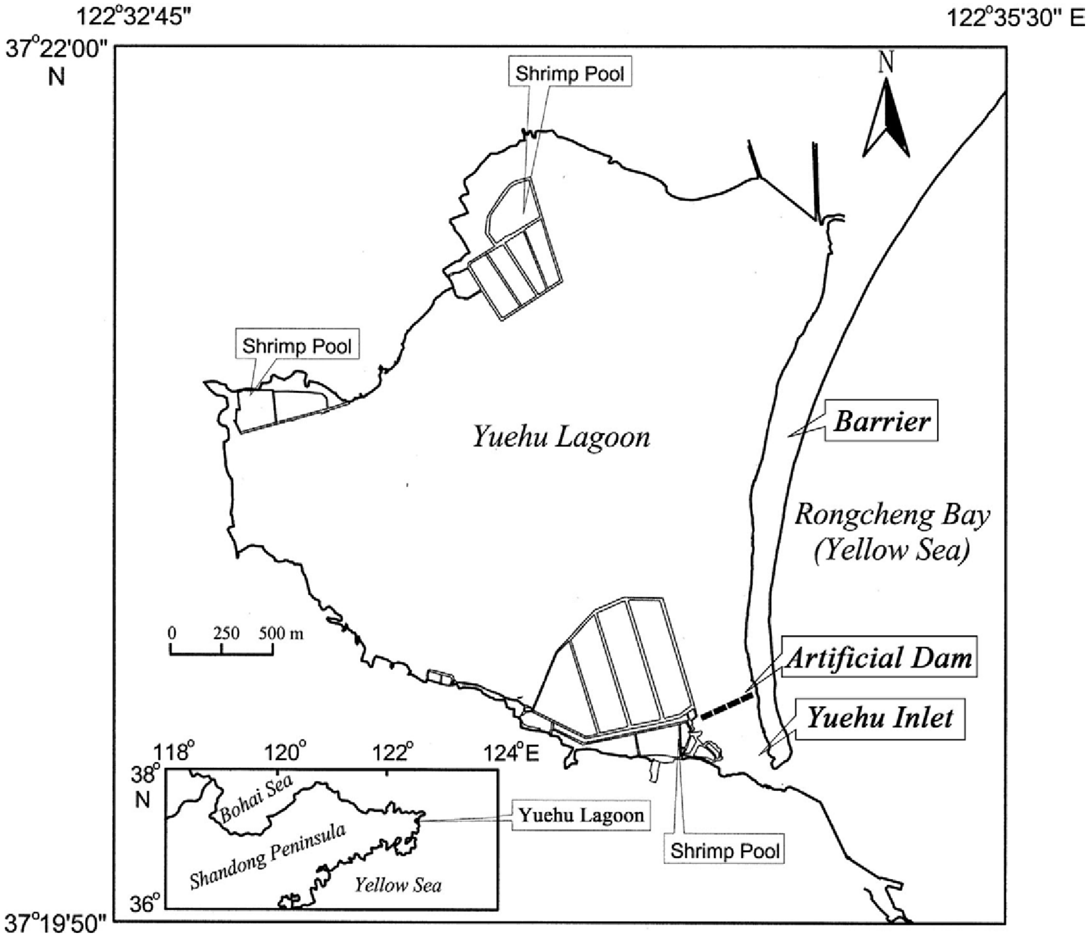

FIGURE 18.1

Map showing the location of Yuehu lagoon in Shandong Province, China, where an artificial dam was built in 1979.

From Jia et al., 2003

production of *A. japonicus* declined sharply, down to 150 kg in 1982 (Gao, 1998). Local residents realized the mistake and tried to remove the dam (at least partially) to restore the environment, but it did not work, as the base of the dam remains in place. Consequently, efforts were made to develop aquaculture differently, and 0.4 km^2 of land was turned into shrimp ponds, which not only failed to bring economic benefits, but further deteriorated water quality in the lagoon. As a result, the inlet system was transformed from an intertidal habitat into a continuously flood-dominated area (Jia et al., 2003). The production of *A. japonicus* continued to drop, and reached 50 kg in 1996. Because examples like Yuehu lagoon are found everywhere along the coast of China, restoring native environments and maintaining healthy ecosystems have now become a priority.

There is no evidence that the wild stocks of *A. japonicus* in China have recovered from overfishing (Wang et al., 2013; Zhang et al., 2014). In Japan, this species has been managed for a very long time and although there has been at least a 30% decline over the past 30 years based on landings data, current populations seem to be stable (Choo, 2008), but problems are expected in the near future (see Chapter 22 for Japan fishery). In sum, data from the 1960s–1970s to about 2005 show that landings have declined by 80% in Russia, >95% in China, 40% in both Koreas, and ~30% in Japan. Overall, it is estimated that this species has declined by at least 60% throughout its global range over the past 30–50 years (Bruckner, 2006; Choo, 2008; Anderson et al., 2011). Therefore, *A. japonicus* is now listed as Endangered on the IUCN Red List of Threatened Species (Hamel and Mercier, 2013; Purcell et al., 2014).

Overfishing not only threatens a valuable source of income and nutrition, but it also negatively impacts the whole ecosystem through effects on food webs and ecosystem functions (Bascompte et al., 2005; Jackson et al., 2001). In recent years, red tides, green tides, and outbreaks of jellyfishes and sea stars have been associated to some degree with overfishing and habitat degradation (Dong et al., 2010).

18.1.2 POLLUTION

While mariculture has produced great quantities of seafood, it has also generated a great deal of pollution in coastal environments. Suspended aquaculture of filter-feeding shellfish in shallow seas is an important industry. However, large amounts of waste and pseudofeces are produced around these cultures. For instance, in Sishili Bay, daily output of feces or pseudofeces in the order of 162 t (dry weight) are produced by farming medium-sized scallops, *Chlamys farreri*, in summer (Yang et al., 2000; Yang et al., 2001; Yuan et al., 2006; Zhou, 2000). In Sanggou Bay, farms of *C. farreri* over 667 m^2 generate 85.4 t (dry weight) of pseudofeces annually (Mao, 2004). Over time, the accumulation of organic waste increases the seafloor's carbon, nitrogen, and phosphorus contents, decreases the dissolved oxygen, and leads to changes in the biochemical characteristics of the sediment and the structure and composition of associated benthic communities (Ragnarsson and Raffaelli, 1999; Stenton-Dozey et al., 2001). The decomposition of organic materials results in secondary organic pollution and in the deterioration of the environment. Especially in summer when the water temperature is high and oxygen levels are low, bacteria are active and stratification prevents the lower layer of water from mixing with the upper layer, which results in hypoxia. The deterioration of water quality results in a deterioration of the whole environment, which may lead to the mass mortality of aquaculture species (Zhou et al., 2006a; Zhou et al., 2006b).

The deposit-feeding sea cucumber *A. japonicus* could play an important role in reducing pollution originating from aquaculture activities. Sea cucumbers chiefly use oral tentacles to collect food particles. Species with capitulum tentacles are all deposit-feeders, ingesting sediments from the seafloor, whereas species with branched tentacles are suspension-feeders, ingesting seston from the water column

(Holtz and MacDonald, 2009; Graham and Thompson, 2009). Most commercial species of sea cucumber are deposit-feeders in the order Aspirochirotida, whereas a few commercial species on the market are suspension-feeders in the order Dendrochirota (Liao, 1997). Deposit-feeding sea cucumbers, like *A. japonicus*, either ingest surface sediment on muddy or sandy bottoms or collect particles on hard surfaces. They utilize organic matter mixed with the sediment as a food source, mainly comprising microorganisms (benthic diatoms, bacteria, protozoa, algae, or foraminifera) and detritus of animal and plant origins (Choe, 1963; Zhang et al., 1995; Uthicke, 2004) (see Chapter 10 for details on feeding biology).

Many recent studies have highlighted that residual feed and feces of some aquaculture species can play an important role in the nutrition of deposit-feeding sea cucumbers. Hauksson (1979) and Kang et al. (2003) reported that the waste produced by other marine benthic animals, such as the residual feed and feces from the abalone *Haliotis discus*, could be used as food by sea cucumbers (see Chapter 17 on coculture of *A. japonicus*). Moreover, Sloan and Von (1980) and Ramofafia et al. (1997) found that the sea cucumbers *Isostichopus badionotus* and *Actinopyga mauritiana* relied on their own feces to offset insufficient food supply.

18.2 HABITAT ENHANCEMENT

18.2.1 ARTIFICIAL REEF FOR HABITAT ENHANCEMENT

It is becoming urgent to find solutions to improve the current state of coastal environments around mariculture installations to restore and optimize habitats and to develop responsible and sustainable aquaculture practices.

Suspended aquaculture of shellfish provides abundant particulate organic matter as potential food for *A. japonicus*. However, not all habitats are suitable for them, as fishing by trawls removes large amounts of small- and medium-sized rocks that could provide shade and protection. In these locations, additional shelters for *A. japonicus* in the form of artificial reefs could be incorporated to take advantage of the food source provided by mollusc cultures. Artificial reefs are defined as a submerged structure placed on the substratum (seabed) to mimic characteristics of a natural reef (Baine, 2001). Artificial reefs are used around the world as fishery management tools and to restore habitats impaired by human activities (Fabi et al., 2002). They can be used in sea cucumber farming (Figure 18.2), because they can improve, repair, and optimize habitat for some aquatic organisms, such as sea cucumbers, by helping them to feed, reproduce, grow, and develop (James, 1996; Zhang et al., 2011). While this will not restore the habitat to its initial state, it is a tool that can assist its rehabilitation.

Improved catches and valuable high-quality aquatic products like sea cucumber are often associated with artificial reef fisheries. Comparisons of production before and after the deployment of artificial reefs demonstrated important economic benefits. According to Dr Sato of Hokkaido University in Japan, the fish catch in artificial reef fisheries can be increased by 10 kg m^{-3} compared to nearby areas without artificial reefs. In China, in the city of Rongcheng, which is one of the most important aquaculture centers, fish caught in artificial reef fisheries reached a value of USD 20 million in 2007. At the same time, the artificial reefs can play a role in optimizing the physical properties of the habitat (Li, 2007).

A variety of industries have also developed following the deployment of artificial reefs, such as coastal hotedls, restaurants, bait shops, and sport fishing shipyards, and employments in these industries increase by 3–5% annually currently. After the completion of artificial reefs, the fishing and diving areas can also become centers of marine eco-tourism, such as the artificial reefs around Qiansan islets,

FIGURE 18.2

Different types of artificial reefs for sea cucumbers. (A) Multilayer, plate-type sea cucumber reefs. (B) Deploying artificial oyster-shell reefs by boat. (C) Integrated three-floor artificial reefs. (D) Deploying integrated three-floor artificial reefs in coastal waters.

Photo A by Libin Zhang, Photo B by Tao Zhang, Photos C and D by Qiang Xu

Haizhou Bay (Yellow Sea, China). Therefore, the construction of artificial reefs not only produces ecological benefits, but economic benefits as well. In Haizhou Bay, the density of *A. japonicus* in artificial reefs deployed in March 2008 reached 3–5 ind. m^{-2} in May 2009, while stocks of commercial fish (*Lateolabrax maculatus*, *Hexagrammos otakii*, *Sebastes schlegeli*) increased by 25–35% (Zhang, 2010).

Artificial reefs also provide shelter for marine animals against predators (including humans) and unfavorable environmental conditions (e.g., storms, bottom trawling, and sedimentation). They provide a hard substrate to sessile organisms, such as algae and bivalves. It has been reported that a few days after the reefs had been deployed, organisms began to settle on them; algae attached to the structures followed by sessile organisms, e.g., barnacles and oysters (Pickering and Whitmarsh, 1997). These sessile organisms grew rapidly, covering almost the entire surface of the reef after a few months. Sea cucumbers can find an abundance of food sources, such as diatoms, bacteria, protozoa, and algae, whereas increasing plankton and larger benthic organisms can provide food sources for fish. Waters with a rich diversity of prey organisms naturally become a good habitat for sea cucumbers and fish. In turn, the growth of algae consumes some of the excess nitrogen and phosphorus whereas part of the new sediments accumulating on the sea floor are ingested by sea cucumbers, which helps maintain water quality and reduces the incidence of algal blooms (red tides). This is particularly true for artificial reefs installed close to bivalve aquaculture systems. The construction of artificial reefs can enhance both the number of sea cucumber and shellfish, leading to an increase in the number of crustaceans and fish, which can gradually restore the damaged food web. It has been argued that by constructing and implanting artificial reefs, the marine environment of key coastal areas has improved, some rare and threatened or endangered wild animals are more effectively protected, and marine fisheries are becoming more sustainable (Lin, 2011).

18.2.2 ARTIFICIAL REEF DESIGNS FOR *A. japonicus*

In China, many materials have been utilized as artificial substrata or to build reefs for sea cucumber culture, such as stones (Liu et al., 2001; Li et al., 2004; You, 2004; Yang et al., 2005b; Chen, 2005; Sun and Chen, 2006; He et al., 2007; Chen, 2007), tiles (Chen, 2003; Wang et al., 2004; Qin et al., 2009), concrete structures (Zhao, 1995; Sun, 2004; Qin et al., 2009), scallop lantern nets (Li, 2007), woven fabrics (Lin, 2007), plastic components (Li and Huo, 2007), and even Chinese oak branches (Figure 18.2) (Yang and Shan, 2007). Although these substrata were developed and tested, they had irregular shapes that complicated the harvesting of the sea cucumber *A. japonicus*.

Many factors influence habitat selection by animals, such as physical features (type, shape or color of materials), presence/absence of food, presence of potential predators, and competition with other species (Riechert, 1976; Young and Chia, 1982 Abramsky et al., 1990; Ward and Porter, 1993; Hamel and Mercier, 1996; Yan and Chen, 1998; Yang et al., 2005a). Artificial reefs are commonly used for sea cucumber ranching in China (Chen, 2003), as they provide protection against predators (Ambrose and Anderson, 1990), supply food, and provide shelter for aestivation (Zhang and Sun, 2001; Chen, 2005; Chen, 2007; Qin et al., 2009) (see Chapter 11 for details on aestivation).

An increasing demand for beche-de-mer, together with evidence of a world-wide decline in natural stocks of sea cucumbers (Hamel et al., 2001; Conand, 2004; Purcell et al., 2013), suggests that the development of efficient enhancement methods are urgently required to sustain the demand. In this context, Zhang et al. (2011) tested a new culture system, a multilayer plate-type reef, which can be varied in color, shape/style or spacing, and can be used for pond culture of sea cucumbers (see details

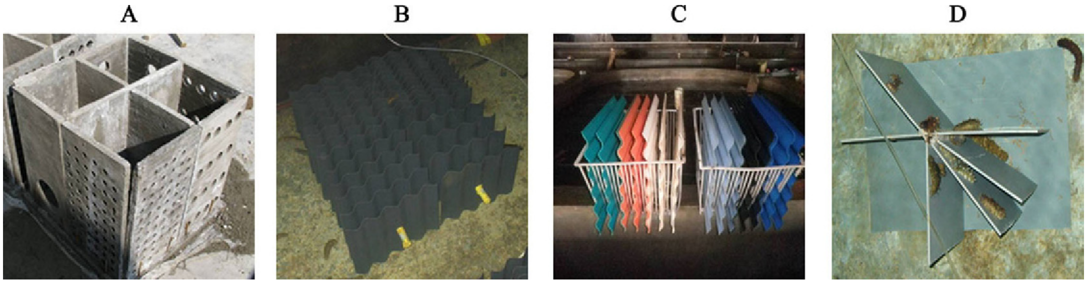

FIGURE 18.3

Artificial reef models. (A) Aperture reef models. (B) Interval spacing reef models. (C) Color reef models. (D) Angle reef models with specimens of *A. japonicus* visible.

Photographs by Chenggang Lin

later). Lin et al. (2012) studied the behavior and selectivity of the sea cucumber *A. japonicus* as key indicators in artificial reef design (aperture, interval spacing, color, angle; Figure 18.3) using the mean attractive rate (MAR) method (Zhang et al.,2006):

$$\mathrm{MAR} = \sum_{i=1}^{n} (N_i / nN) \times 100\%$$

where N_i refers to the quantity of the sea cucumber in each cell at some point in time; N the sum of the sea cucumber; i the point in time, and n the total number of times the experiment was repeated.

Four ecological indicators were defined based on macrobenthos in reef and nonreef areas. The Shannon–Wiener index and Margalef index are common diversity indices (Marques, 2009; Verissimo et al., 2012). These two indices were higher in the reef area than in the nonreef area from November to March.

The M-AMBI index, combined with AMBI, Shannon–Wiener index, and species richness, was higher in the reef area than in the nonreef area. According to the standard of M-AMBI (Borja et al., 2007), the reef area was in good condition from September 2010 to May 2011, whereas the nonreef area was in moderately good condition from November 2010 to May 2011. Those results indicate that the environmental quality of the oyster reef area was slightly better than that of the nonreef areas.

18.2.2.1 Attraction of sea cucumbers to different colors

Animals select suitable habitats for protection against predators and/or competition and food availability, among other things (Yan and Chen, 1998). This selectivity includes matching body color with background color for concealment (Zhou et al., 2000). Zhang et al. (2009) have demonstrated that black and gray settlement substrata are more desirable to *A. japonicus* than red, white, green or yellow substrata under laboratory conditions. A later study (Lin et al., 2012) showed that *A. japonicus* tended to settle on darker-colored material that is most similar to their own body color (Figure 18.4). Thus, the number of animals in red, blue, and black structures were higher than in green or white structures. The number of animals in transparent structures was the lowest, as they provided little shade for sea cucumbers. This means that darker materials should ideally be used in the design of artificial reefs (Zhang et al., 2009a, 2009b; Lin, 2011).

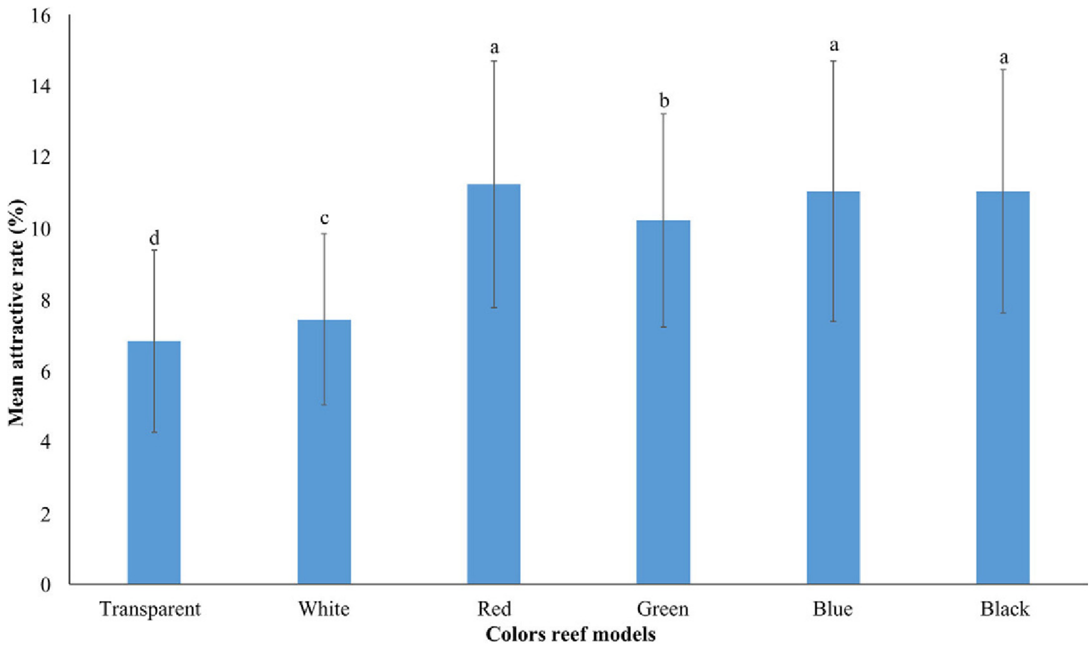

FIGURE 18.4

Attractive rate of *A. japonicus* to different colors of reef models. Means with different letters denote significant differences ($P < 0.05$), and bars represent S.D.

From Lin, 2011

18.2.2.2 Attraction of sea cucumbers to different spacings

A. japonicus tends to inhabit crevices and holes in rocky reefs that provide shade and protection against predation (Zhang et al., 1995). Also, *A. japonicus* cannot tolerate high temperatures. It is a temperate sea cucumber that is known to aestivate when water temperatures rise above a certain threshold level (Yang et al., 2006; An et al., 2007; Yuan et al., 2007; Dong et al., 2008; Ji et al., 2008; Wang et al., 2008; Yuan et al., 2009) at which time they hide in crevices (see Chapter 11 for details). Zhang et al. (2011) showed that adult sea cucumbers (>10 g ind.$^{-1}$) preferably selected crevices with 2-cm-wide openings (Figures 18.5 and 18.6). It was suggested that the spacing offered by artificial reef designs should be 2 cm.

18.2.2.3 Attraction of sea cucumbers to different shapes/styles

Zhang et al. (2006) found that *A. japonicus* individuals exposed to high light intensity either migrated to the shaded area created by the presence of artificial reefs or directly hid inside them. Zhang et al. (2006) also showed that varying the angle of the corrugated sheets provided different degrees of shading, with greater shades created by oblique than either parallel or wavy designs. It might be the reason why the numbers of *A. japonicus* found in the 15° design were higher than in the other tested designs (Figure 18.7), suggesting its suitability for artificial reefs designed for *A. japonicus*.

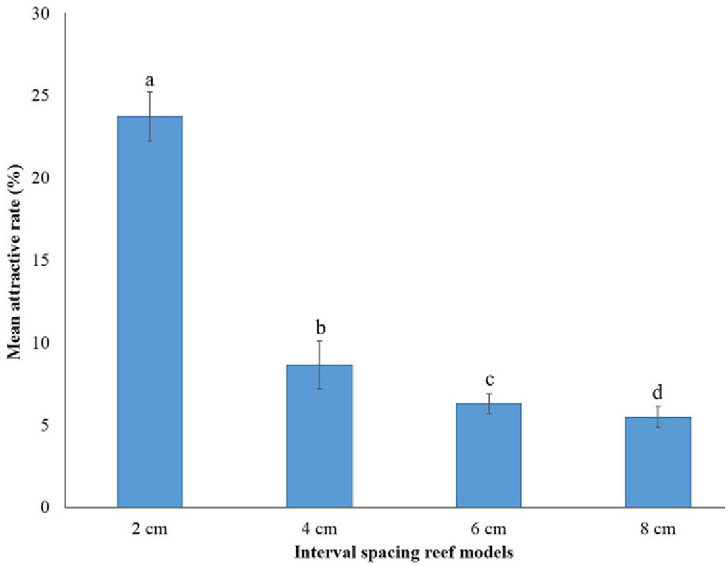

FIGURE 18.5

Attractive rate of *A. japonicus* to various spacing reef models. Means with different letters denote significant differences ($P < 0.05$), and bars represent S.D.

From Lin, 2011

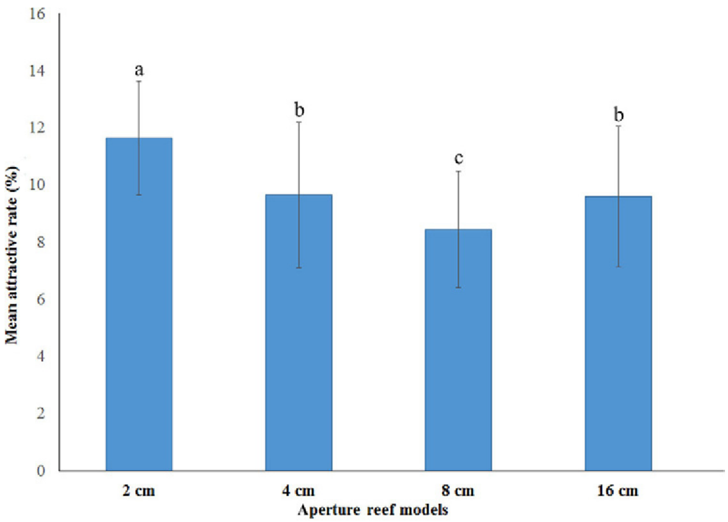

FIGURE 18.6

Attractive rate of *A. japonicus* to various aperture reef models. Means with different letters denote significant differences ($P < 0.05$), and bars represent S.D.

From Lin, 2011

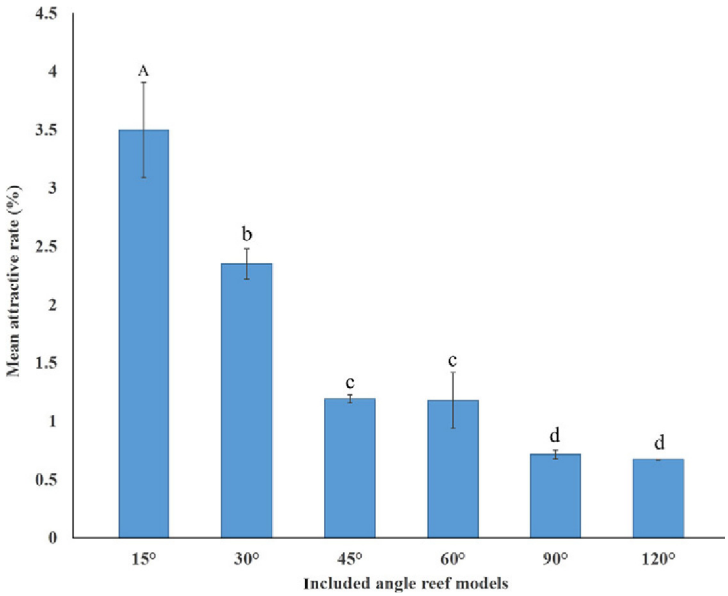

FIGURE 18.7

Attractive rate of *A. japonicus* to various angle reef models. Means with different letters denote significant differences ($P < 0.05$), and bars represent S.D.

From Lin, 2011

18.2.2.4 Vertical distribution of sea cucumbers

In the natural environment, *A. japonicus* lives in crevices among rocks close to or directly within seagrass beds (*Zostera marina*), preferably in areas with low light intensity. When the water is poorly oxygenated close to the substrate, sea cucumbers tend to climb onto *Zostera* leaves or rocks to position themselves higher in the water column, farther away from the bottom boundary layer. Zhang et al. (2011) showed that from March to May (high activity period), *A. japonicus* climbs to the "upper layers" of the reef structures, most likely in order to have access to better-oxygenated areas, especially in cofferdams.

18.2.2.5 Oyster-shell reefs for sea cucumbers

Many materials, such as tires and concrete blocks, can be used to construct artificial reefs. Oyster shells are an abundant and readily available waste product of the food industry in Shandong Peninsula (China), which can also be used. Artificial reefs composed of oyster shell bags are believed to provide an appropriate habitat for *A. japonicus* (Xu et al., 2014) because they offer an irregular substrate that may retain suspended material, providing food sources for deposit-feeding sea cucumbers (Figure 18.8).

Xu et al. (2014) deployed oyster-shell reefs in June 2009 in Rongcheng Bay off the east coast of Shandong Peninsula. This type of artificial reef consists of piles of oyster shell bags placed 2–3 m apart on the seabed. Each pile weighs about 100 kg and is 1 m high. The bag piles do not totally cover the seabed, leaving expanses of mud between them. Xu et al. (2014) revealed that Chl-*a* was different between reef and nonreef areas, but other factors (TOM, TOC, and TN contents), though slightly higher

FIGURE 18.8

Artificial oyster-shell reef, showing bags filled with oyster shells to be deployed.

Photograph by Qinzeng Xu

in the artificial reef, were not statistically different between the two areas. There are several possible explanations for this, including the small size of the artificial reef and short distance between the two areas compared. The sedimentary processes in the study site were influenced by kelp growth.

18.3 HABITAT REHABILITATION

18.3.1 SEAGRASS BEDS

Native habitats where *A. japonicus* could be found are characterized by abundant rock cover, and often the presence of seaweed and seagrass (Liao, 1997; Zhang et al., 1995). The abundance of seagrass has decreased greatly in China since the 1970s, owing to human-induced habitat deterioration and the low economic value of seagrass itself (Zhou et al., 2014). Moreover, until recently, the role of seagrass or derived detritus as a potential food source for sea cucumbers had not been determined. Liu et al. (2013) showed that seagrass-generated detritus could be used as a food source for *A. japonicus.* Mixtures of *Z. marina* debris and sediment were used to prepare five diets. Specific growth rate (SGR) and fecal production rates (FPR) of *A. japonicus* in the five treatments were significantly different (Figures 18.9 and 18.10) (Liu et al., 2013):

$$\begin{aligned} \mathrm{SGR} &= 100(\ln \mathrm{W}_2 - \ln \mathrm{W}_1)\mathrm{T}^{-1}; \\ \mathrm{FPR} &= \mathrm{F}/\mathrm{T}/\mathrm{N}; \end{aligned}$$

where W_1 and W_2 are initial and final wet body weight of *A. japonicus*, T the duration of the experiment, F the dry weight of feces, N the number of *A. japonicus*.

The study showed that a mixture of decaying *Z. marina* debris and marine muddy sediments with an organic content of 19.6%, could lead to a better growth rate for sea cucumbers compared to many other food items or diets tested. Hence, transplantation of seagrass shoots to appropriate habitats contributes to their rehabilitation and helps restore favorable habitats for sea cucumber (Zhou et al., 2014).

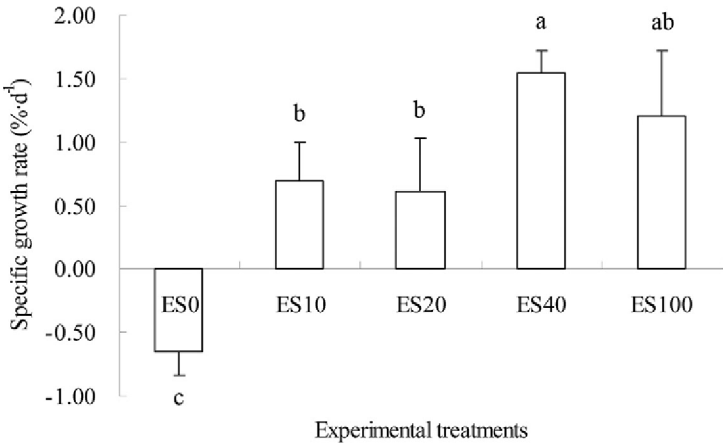

FIGURE 18.9

Specific growth rates (SGR; %·d^{-1}) of *A. japonicus*. Five diet treatments were designed, i.e., ES0, ES10, ES20, ES40, and ES100, with eelgrass debris accounting for 0, 10, 20, 40, and 100% in dry weight, respectively. Different letters denote significant differences ($P < 0.05$), and bars represent S.D.

From Liu et al., 2013

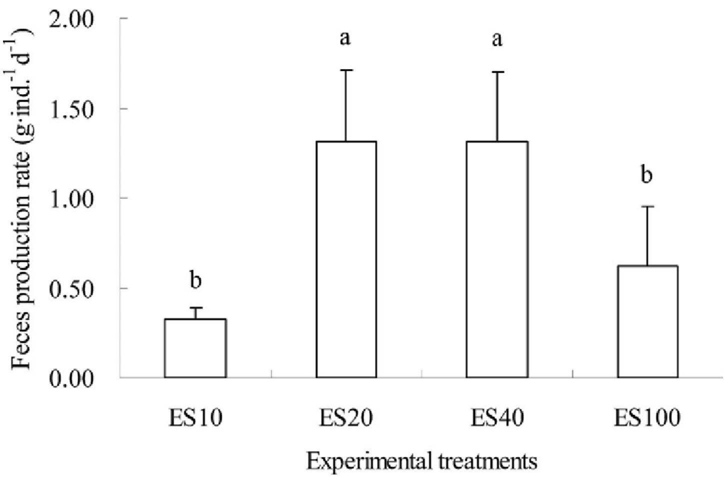

FIGURE 18.10

Fecal production rates (FPR; g ind.$^{-1}$ d^{-1}) of *A. japonicus* during the experimental period. Five diet treatments were designed, i.e., ES0, ES10, ES20, ES40, and ES100, with eelgrass debris accounting for 0, 10, 20, 40, and 100% in dry weight, respectively. Different letters denote significant differences ($P < 0.05$), and bars represent S.D.

From Liu et al., 2013

A total of 22 seagrass species are distributed along China's coastal regions that belong to 10 genera and four families, and account for about 30% of known seagrass species found worldwide (Zheng et al., 2013). The total distribution area for China's seagrass meadows is estimated to be 8765 ha and decreasing (Shi et al., 2010). Planning for seagrass bed restoration is urgently needed at present. There are various types of transplantation being tested: sods, plugs, and shoots with rhizomes. The condition of both the seagrass source and the restoration site, as well as the purpose of restoration should be taken into account when choosing a restoration method. Considering the current situation in China, transplantation of seagrasses is the preferred means of restoration, especially with the idea of increasing suitable habitats for natural populations of sea cucumbers.

18.3.2 *Zostera marina*

The seagrass *Zostera marina* (commonly called eelgrass) is a perennial marine angiosperm belonging to the class Monocotyledoneae and order Helobiae. It is distributed worldwide in the intertidal and subtidal zones of shallow seas, where it grows in muddy or sandy substrata, generally in areas with reduced flow and good water transparency. *Z. marina* occurs in the temperate coastal areas of Shandong, Hebei, and Liaoning provinces in northern China (Yang, 1979). A greater understanding of the importance of seagrass in ecosystem functioning has developed in recent years, spurring an increase in research in China (Guo et al., 2000; Yu et al., 2009; Li et al., 2010). Swan Lake is a small cove located in Weihai, northern China, where the seagrass beds are now expanding due to effective conservation and restoration measures. The sea cucumber *A. japonicus* was found to reach high densities in these areas (Figure 18.11). However, *A. japonicus* was much more abundant 30 years ago, when it covered nearly the whole cove; and there are vivid descriptions of the abundance of sea cucumbers in local historic accounts, i.e., several individuals in one step, evoking extremely high densities of ~10–30 ind. m^{-2}.

In natural eelgrass meadows, deposit feeders can take advantage of organic detritus from eelgrass decomposition, which not only accelerates the cycling of matter, but also promotes the health and stability of the ecosystem structure. Eelgrass meadows in Shandong coastal waters to depths of 2–6 m have deteriorated badly, to the point that some have disappeared entirely (Li and Li, 2006). For the

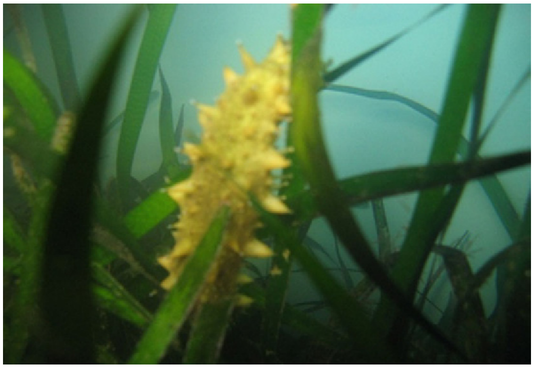

FIGURE 18.11

A sea cucumber *A. japonicus* among seagrasses at 2 m depth in Yuehu inlet.

Photograph by Tianlong Qiu

restoration of the declining natural resource of *A. japonicus*, it is suggested that the degraded eelgrass meadows should be restored in the northern inshore areas of China. Zhou et al. (2014) developed a simple new transplanting technique for *Zostera marina*. To assist in anchoring single shoots, several rhizomes of rooted shoots were bound to a small elongate stone (50–150 g) with biodegradable thread (cotton or hemp), and then the bound packet was buried at an angle in the sediments at a depth of 2–4 cm. This stone anchoring method was used to transplant eelgrass in early November 2009 and late May 2010 in Huiquan Bay, Qingdao. The results were promising. Survivorship of the transplanted shoots was >95% after three months, and the transplanted eelgrass beds matched nearby natural beds in terms of shoot height, biomass, and seasonal variations.

18.3.3 DWARF *Zostera japonica*

The dwarf or Asian eelgrass, *Zostera japonica*, is a perennial marine flowering angiosperm that is mainly distributed in the intertidal and shallow subtidal zones from temperate to subtropical regions along the North Pacific coast, especially in East Asia (Short et al., 2007).

Many studies have focused on the distribution and impact of this invasive species. Generally, intertidal *Z. japonica* competes with indigenous seagrasses only to a limited extent, and thus its invasion has minimal negative impact on the local ecosystem (Williams, 2007). Moreover, dwarf eelgrass converts the intertidal mud flat into a seagrass bed, thereby increasing primary productivity (Kaldy, 2006; Ruesink et al., 2006) and enriching the local marine biodiversity (Posey, 1988).

A number of new reports on the ecology of dwarf eelgrass have been published (Lee, 1997; Lee et al., 2005, 2006a, 2006b; Abe et al., 2010; Kishima et al., 2011; Morita et al., 2011), including in southern China (Fan et al., 2011). Recently, Zhang (2013) studied *Z. japonica* in a temperate lagoon in northern China to document the ecological features and growth status of dwarf eelgrass in northern China. The adaptation of *Z. japonica* to the intertidal environment was also evaluated.

Despite the introduction of *Z. japonica* to the North American Pacific Coast and its continued expansion, the seagrass in its original habitats (i.e., temperate and subtropical areas of the western Pacific Coast) has undergone severe decline (Huang et al., 2007; Abe et al., 2010). The subtropical coast of southern China is also facing great challenges (Fan et al., 2011). However, in the intertidal zone of Swan Lake in Rongcheng, *Z. japonica* is widely distributed and even forms an extensive seagrass bed. *Z. japonica* on the east coast of Swan Lake began to spread in June–July 2012. Before then, such an expansion had never been seen in this area. The eelgrass coverage also increased rapidly, reaching as high as 100% in August 2012. Based on GPS measurements, the distribution area of the dwarf eelgrass bed was estimated to be $\sim 2.7 \times 10^4$ m^2.

In addition to favorable environmental conditions, the establishment of the Swan National Nature Reserve in China has contributed to the survival and expansion of *Z. japonica* in Swan Lake, thereby providing sea cucumbers with a suitable habitat. The environment in the lagoon is well protected and free from human interference.

18.4 CONCLUSION

It is becoming increasingly clear that artificial reefs can enhance the survival and growth of *A. japonicus*. New structural designs were shown to provide higher yields than the altered environment without reefs. For *A. japonicus*, black, corrugated plastic sheets with a 2 cm spacing and oblique orientation

emerge as the most attractive design for culture and stock enhancement in cofferdams or ponds. Multilayer plate-type reef structures, oyster-shell reefs in particular, can positively enhance the farming/habitat space availability. Efforts to rehabilitate seagrass beds through transplantation methods are also yielding promising results, with the potential to create suitable habitats for *A. japonicus*.

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CHAPTER

19 NUTRITIONAL AND MEDICINAL VALUE

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SUMMARY

Sea cucumber has been regarded as a highly nutritional food since ancient times, giving it a high economic value today, and making it one of the "eight valuable seafoods" in China. The most prized species in China, *Apostichopus japonicus*, is high in protein, low in fat, and it contains an abundance of trace elements and other inorganic salts. Furthermore, it is a great source of functional molecules, such as polysaccharides, collagen proteins, and lipids, among others. This chapter introduces the nutritional value of *A. japonicus* and the composition and function of its major bioactive molecules.

Keywords: *Apostichopus japonicus*; bioactive compounds; collagen; lipids; nutritional value; polysaccharides; sea cucumber

19.1 ACTIVE INGREDIENTS OF SEA CUCUMBER

The body wall is the sea cucumber's major edible (and medicinal) organ. It consists mainly of epithelial and dermal connective tissues. Among the general biochemical components (related to nutrition), moisture content stands the highest, followed by crude protein, ash content and total carbohydrate, and finally crude fat content. The body wall of sea cucumber also contains polysaccharides, lipids, and collagen (see Chapter 13 for details on tissue biochemistry). The active ingredients in sea cucumber are believed to allay fatigue, boost the immune system, strengthen resistance to disease, treat injuries, prevent inflammation, and provide liver and blood vessel protection, among many other properties. Active nutritive ingredients in sea cucumber play an important role in health care in China.

19.1.1 POLYSACCHARIDES

Polysaccharides account for >6% of the total organic contents of sea cucumber (Katzman and Jeanloz, 1970). The polysaccharides found in the body wall are mainly divided into two categories (Yoshida et al., 1992): one is holothurian glycosaminoglycan (HGAG), consisting of branched heteropolysaccharides composed of D-*N*-acetyl galactosamine, D-glucuronic acid, and L-fucose, with a relative molecular weight of 40–50 kDa; the other is holothurian fucan (HF), which is an unbranched polysaccharide

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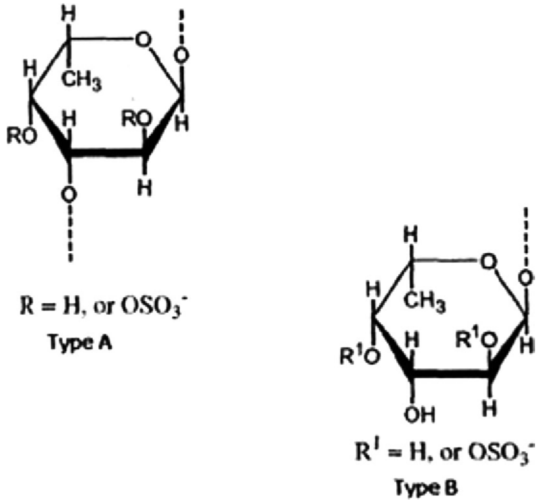

FIGURE 19.1

Hypothetical structure of two types of fucan from *A. japonicus*.

From Kariya et al., 2004

composed of L-fucose, with a relative molecular weight of 80–100 kDa. Although their glycosyls are different, their partial hydroxide radicals on the carbohydrate chain are sulfated, and the sulfated polysaccharide percentages of both are about 32%; their polysaccharide structure are unique to all sea cucumbers. Sea cucumber polysaccharides feature unconformity in sulfating degrees, heterogeneity in microstructures and molecular weights, and variety in residue amount, which results in complexities in the purification and structure analyses. Kariya et al. (2004) separated and purified holothurian glycosaminoglycan from *Apostichopus japonicus* (Figure 19.1). After acidolysis, its monosaccharide and acidophobe composition were measured revealing an E-type structure of chondroitin sulfate. The molar ratio of acidophobe (SO_4^{2-}), galactosamine (Gal), glucuronic acid (GlcUA), and fucose (Fuc) was 3:2:2:1. It was shown by further structural analyses that the fucose branched chain of this chondroitin sulfate was connected to the 0–3 loca (20%) of the glucuronic acid branch by two fucoses with 1*3 glycosidic bonds, and the residual fucose branched chain may be connected to 0–4 and /or 0–6 (60, 10, and 10%) loca of N-B phthalein galactosamine. Sheng (2007) took *A. japonicus* and *Holothuria mexicana* as crude materials. After treatments, such as enzymolysis with papain, CPC sedimentation, alcohol precipitation, ultrafiltration, and desalination, were carried out on crude materials, extracts were obtained that had molecular ratios of amino sugar, uronic acid, fucose, and sulfate radical of 1:1.71:2.57:6.79 and 1:1.12:1.98:5.25, respectively, and percentages of fucose, galactose, glucose, and mannose of 12.67 and 15.58%, 1.56 and 2.01%, 6.19 and 0.42%, 0.55 and 0.38%, respectively. Wang et al. (2012) reported that a nortriterpene glycoside, 26-nor-25-oxo-holotoxin A1 (1), four triterpene glycosides, including both holostane and nonholostane type analogues, holotoxins D–G (2–5), together with three known triterpene glycosides, holotoxins A1 and B (6, 7), and cladoloside B (8), were isolated from the warty sea cucumber *A. japonicus* Selenka, a traditional tonic with high economic value in China (Figure 19.2).

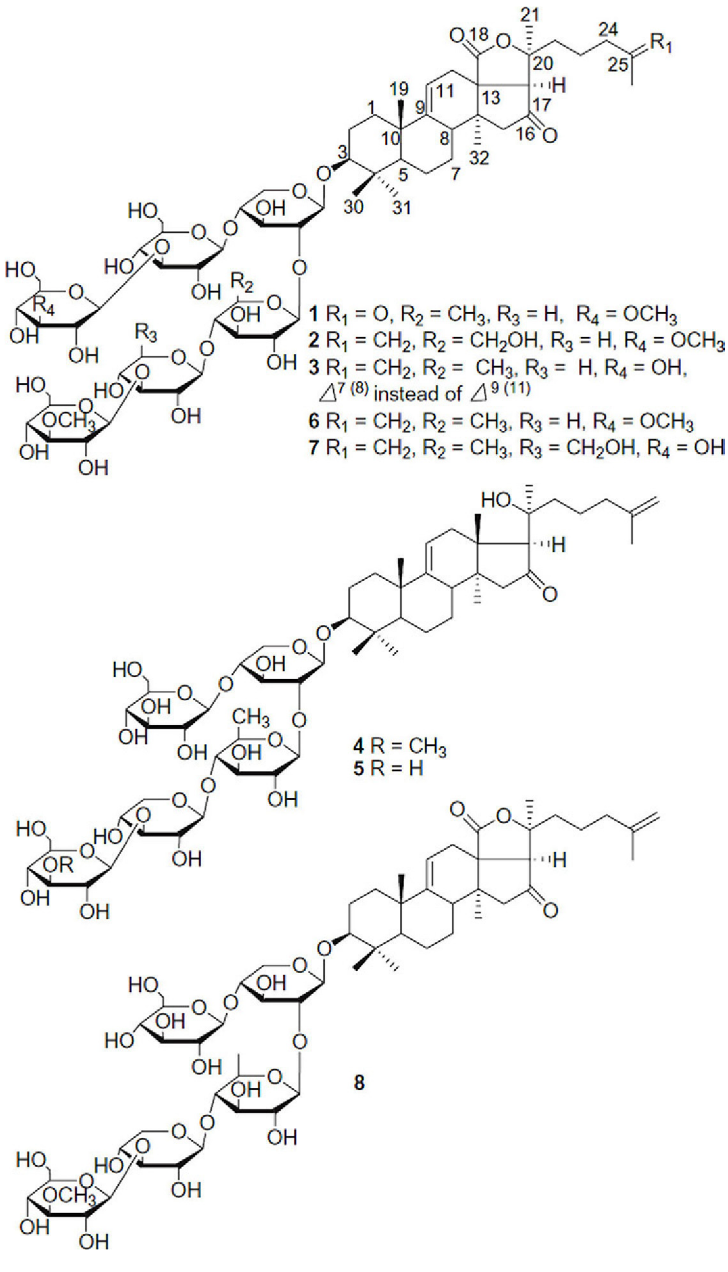

FIGURE 19.2

Hypothetical structure of glycoside from *A. japonicus*.

From Wang et al., 2012

Compared with sea cucumbers, *A. japonicus* in particular, the total content of sulfated polysaccharides found in other traditional Chinese medicines, such as donkey-hide gelatin (glue), deer antler glue, tortoise plastron, and shell glue, which are also made of animal connective tissue, takes up only 0.2%. In addition, the content of sulfate glycosaminoglycan in top-grade sika deer velvet is equal to that of sea cucumber. However, the content of its sulfate-containing polysaccharides is <15% as well. The polyanion content in top-grade shark fins is close to that of Grade 1 spotted-deer antlers. Incidentally, both bird's nest, which is recommended in traditional Chinese medicine for tonifying *qi*, and chicken's gizzard-membrane, which is excellent for invigorating the spleen and relieving dyspepsia, also contain many polyanions and acid glycoproteins (Gao, 2008).

Kalinin et al. (2007) point out that the biological activities of holothuroid glycosides that include strong ichthyotoxic action and physical properties indicate important external defensive function against predators. Over the past 20 years, extensive pharmacological investigations on sea cucumber polysaccharides, especially HG and depolymerized HG (DHG), have verified and confirmed its numerous properties: (1) HG can reduce the growth and metastasis of tumors in a variety of experimental animals (Guan and Ding, 1999). (2) HG can improve the immunity of cells, improve and strengthen compromised immunity caused by cancers or anticancer drugs (Li et al., 1985; Chen et al., 1987; Sun et al., 1991). (3) HG can prevent neovascularization, including that caused by transplanted tumors; at the same time, cortisone can intensify the inhibiting effects of HG on neovascularization (Liang and Wei, 1998; Hu et al., 1997). (4) As a new anticoagulant, HG plays various roles during the coagulation process. Although in normal circumstances, the anticoagulating effect of HG is weaker than that of heparin, its active mechanism (i.e., independent from AT-III) and safety (i.e., lower hemorrhagic tendency) are superior to those of heparin (Nagase et al., 1996, 1997a, 1997b). The anticoagulant mechanism of HF is similar to that of the new anticoagulant and antithrombotic drug dermatan sulfate (Pereira et al., 1999). (5) HG can promote the dissolution of fibrous protein by activating plasminogen; HG can also restrain polymerization among monomers of fibrous protein and change the conglomeration texture of fibrous protein to facilitate fibrinolysis through medication (Yang et al., 1990). (6) In models of venous embolism and acute pulmonary embolism in mice, HG can restrain the formation of embolism and improve survival rate (Nagase et al., 1997a, 1997b). The effects of HG in treating disseminated intravascular coagulation and preventing thrombosis were proven in preliminary clinical tests (Wu et al., 1985; Li et al., 2000). (7) HG can restrain proliferation of vascular smooth muscle cells (VSMC), improve the proliferation and migration of endothelial cells, and stimulate endothelial cells to release free TFPI (tissue factor pathway inhibitor). Thus, it has the potential to become a new therapeutic agent for artery thrombosis and restenosis (Tapon-Bretaudière et al., 2000). (8) In tests with middle-aged and aged people (48–72 years old), HG can reduce blood viscosity and plasma viscosity; it can also regulate blood fat, i.e., reducing serum cholesterol and triacylglycerol and increasing apolipoprotein A while reducing the apolipoprotein B (Li et al., 2000). (9) HG has obvious protective effects on damage or apoptosis of cortical neurons caused by amyloid B protein to rat cortical neurons *in vitro*. It is inferred that this polysaccharide may help prevent degenerative diseases of central neurons, such as Alzheimer's disease (Qiu et al., 2011). (10) HG can prevent specific pathologies of cultured tissue cells caused by herpes simplex virus HSVs (Chen et al., 1980); HF can also greatly inhibit the infection rate of cultured cells by HIVs. In addition, preliminary clinical tests have verified that HG exhibits greater efficacy than conventional drug treatment in terms of restoring liver function of chronic hepatitis patients, and turning "three positive symptoms" (namely HbsAg, HbeAg, and HBcAg) into negative level (Hoshino, 1992).

(11) It has radioresistant activity, i.e., protective effects for animals exposed to Cobalt-60, and can boost the recovery of hematopoietic function. (12) Finally, its anti-inflammatory action may be used as a therapeutic agent against osteoarthritis (Collin, 1998).

19.1.2 BIOACTIVE LIPIDS

The lipid content of sea cucumber is relatively low, accounting for 4% of the dry weight in *A. japonicus* (Li et al., 2006). It mainly includes fatty acids, pigments, sphingolipid, and sterol. Phospholipids account for 90% of the total lipid content of *A. japonicus*. Cholesterol is low, accounting for 1% of total lipid content. Sterols and sphingolipids are the most important bioactive lipids in sea cucumbers; they include free sterol, mixed sterol, and saponin. Sterols are corticosteroids, which are very common in living organisms. The Δ5 sterol content in the sea cucumber body wall is extremely low (Stonik, 1999). There are dozens of different kinds of sterols, mainly Δ9 and Δ7 sterols. Sea cucumber saponin is a polar lipid, which belongs to the triterpenoid oligosaccharide of lanosterol type in chemical structure. The structure of sea cucumber saponin is unique (Minale and Palagiano, 1996), i.e., 3β-20α-dihydroxy-lanostane-18-carboxylic acid lactone. Research indicates that there is also the isomer of 18-carboxylic acid lactone (Sullivan, 1955) and non-sea cucumber stanol triterpenoidal aglucone in *A. japonicus* (Kaneko et al., 1999). According to the difference between the structure of steroid rings and side chain, it has been confirmed that there are more than 50 types of sea cucumber saponins. There are also about 30 types of oligose carbohydrate chains, of which the sequence has been clarified. Sea cucumber cerebroside is the ingredient with the highest content of glycosphingolipid in the sea cucumber body; it was identified as glucose cerebroside through structural analyses. Furthermore, another series of glycosphingolipid – gangliosides – was separated from sea cucumber *A. japonicus* (Xu, 2011). Long chain base (LCB), which is also called sphingoid base or sphingoid, is the characteristic structure of glycosphingolipid, and belongs to the simplest functional sphingolipid compound. Sphingolipid and sterol are the most important active lipids in sea cucumber. They serve various bioactive functions, such as tumor inhibition, immunological competence adjustment, antivirus, antibiosis, and anti-inflammation. Compounds can be formed by combining sea cucumber saponin and sterol molecules on biological membranes, so as to form water stoma and single ion channels, causing the dissolution of the biological membrane. When the concentration of sea cucumber saponin is low, K^+ can leach from lysed cells, which influences the transferring function of cell membranes, or even leads to cell destruction. When saponin concentration is high, amino acids and nucleotides would leak out, causing cell dissolution. This theory is the base of a series of psychological and pharmacological activities. Sea cucumber holothurin A separated from *Actinopyga agassizi* in 1952 is the first recognized animal saponin (Nigrelli, 1952). Holothurin A is the first animal ingredient found to inhibit tumors (Sullivan et al., 1955). Through wide screening of pharmacological models, several types of sea cucumber saponin with different structures have been discovered with biological and medical functions as follows: (1) Cell toxicant action on experimental tumor cells (including human tumor strains) (Sullivan et al., 1955; Miyamoto et al., 1990), the inverse ratio prototype saponin of some acidophobe released from the deoligosaccharide chain is more active. (2) Growth inhibitor of malignant bacteria (Gram positive and Gram negative) and pathogenic fungi (e.g., *Trichophyton*, *Candida*, *Aspergillus*, and *Trichomonas*) (Samoilov and Girshovich, 1980; Miyamoto et al., 1990); holothurin A is particularly effective at killing molds. (3) Prevention of tissue cell oxidation (Rowe, 1969). (4) Immunomodulatory effects and immunological competence of

collaborative vaccine (Sedov et al., 1984; Sharova et al., 1991). (5) Antigen activity (Nigrelli et al., 1967). (6) Growth stimulation of marrow blood cells (Brekhman, 1970). (7) Quasi-estrogen activity, which can inhibit ovulation and stimulate uterine contraction (Mats et al., 1990). (8) Blocker of neuromuscular transmission, used to prevent brain paralysis and spasm caused by cerebral concussion and vertebra damage (Bunch, 1971).

19.1.3 BIOACTIVE COLLAGEN

The sea cucumber's body wall contains a variety of amino acids, which could be comparable to egg protein (Li et al., 2006). Glutamic acid content is the highest, followed by glycine; furthermore, serine, aspartic acid, arginine, and proline contents are relatively high while cysteine and tryptophan contents are relatively low. *A. japonicus* protein mainly consists of collagen (Matsumura, 1973), the ingredient and function of which can be compared favorably with that of traditional Chinese medicine donkey-hide gelatin, glue of tortoise plastron, turtle shell, and deerhorn glue (Fan, 2001). Collagen in the sea cucumber body wall accounts for 76.5% of the total protein content. Sea cucumber collagen has yet to be fully characterized. Type I and V collagens are the most widely reported in aquatic invertebrates, which is similar to Type I collagen existing in vertebrates. Matsumura (1973) pioneered research on *A. japonicus* collagen. Based on amino acid composition (19-1), sea cucumber collagen is similar to that of other echinoderms, and therefore different from that of humans. The analysis of Gly-*X*-*Y* triplet peptide fragment degradation separated from zymolyte of sea cucumber collagen (Fan, 2001) indicates that hydroxylysine through glycosyl is positioned at *Y*; the glycopeptides connection section in collagen molecule is rich in proline, glutamic acid, and alanine residues. The high glycine content of sea cucumber collagen after processing and the presence of hydroxyproline and hydroxylysine also confirm that the major ingredient of sea cucumber protein is collagen. Glycine and basic amino acids in animal collagen or gelatin are responsible for hematogenesis, nourishing blood (biosynthesis of heme and globin), and helping calcium absorption (Pan, 2001). In sea cucumber collagen, besides the high content of lysine and arginine, there is also high content of tryptophan, which increases the nutritive value of the single collagen ingredient (or gelatin). Isemura et al. (1973) obtained some substances of hydroxide radical lysine glycopeptides. The hydroxide radical lysines through glycosyl are all positioned at *Y* of Gly-*X*-*Y* tripeptide, which is also distributed in the whole peptide chain. It is different from the collagen found in the skin of vertebrates. The structure, composition, functions, and characteristics of animal collagen fibers varies. Echinoderms have connective tissue with variant mechanical characteristics, which are controlled by the nervous system. Trotter et al. (1995) pointed out that the length of collagen fiber in sea cucumber is between 30 μm and 1 mm with a symmetric fusiform shape. The ratio of average length to diameter of fiber is ~2000–2500. The collagen fiber of echinoderms is a bipolar molecule. The amino terminal in all molecules face the nearest fiber end, which is placed in antiparallel structure. Kariya et al. (1997) pointed out that there is mucopolysaccharide (GAGs) in the gaps of *A. japonicus* collagen fibers. The tissue consisting of these fibers exhibits variable mechanical characteristics, and is controlled by the nervous system. The collagen fiber of echinoderms is quite similar in structural and functional characteristics. Two kinds of glycoproteins (PGs) were separated from the dendrochirote sea cucumber *Cucumaria frondosa* by Trotter et al. (1995). The degeneration of collagen and the destruction of disulfide bond are essential for separating these two PGs from collagen, which indicates that one or more disulfide bonds play an important role in combining PGs and collagen. Sea cucumber dermis is one kind of collagen tissue. Although collagen fiber is shorter than collagen tissue

without a permanent connection between different fibers, the difference of these tissues is relevant to characteristics of collagen. Due to the function of different protein factors released by the neurosecretory cells, the mechanical characteristics would change fast and be reversible. For example, Trotter et al. (1995) pointed out that stiparin is one kind of structural glycoprotein in the extracellular matrix of the sea cucumber body wall, which may cause the congregation of collagen fiber. Under nondenaturing conditions, it is measured through precipitation equilibrium analysis that its molecular weight is about 375 kDa, while the molecular weight is about 350 kDa by polyacrylamide gel electrophoresis. Trotter et al. (1995) pointed out that tensilin is a collagen-fiber-binding protein existing in the dermis of the sea cucumber, which can be combined with stiparin, so as to disable its ability of congregating collagen fiber (Table 19.1).

There are various kinds of biologically active peptides in the sea cucumber body wall with vital functions. The content of biologically active peptides *in vivo* is extremely low, but its biological activity is extremely high. It has various important biological effects and physiological functions, which

Table 19.1 Composition and Levels of Amino Acids in the Body Wall of Wild *A. japonicus* (mg g^{-1} dry weight) Collected from Zhangzi Island (China) in Different Seasons.

Amino Acids	January	May	August	November
Asp	18.37	22.01	29.90	19.30
Glu	28.92	41.94	64.40	35.50
Ser	10.86	11.66	0.00	102.00
Gly	32.91	23.05	46.10	26.60
Thr	14.92	15.63	19.20	9.60
Arg	25.05	21.96	27.10	13.90
Pro	21.10	20.82	25.80	13.60
Ala	12.39	10.68	21.00	12.10
Val	12.32	10.53	16.90	7.80
Met	5.36	8.26	7.00	3.00
Cys	—	—	1.40	0.60
Ile	12.45	9.98	12.40	6.00
Leu	13.43	10.64	9.10	8.70
Phe	15.07	11.86	1.70	6.70
His	4.14	6.83	2.10	1.70
Lys	5.12	7.23	22.00	11.30
Tyr	9.16	10.98	5.40	3.50
Total amino acid	241.56	234.08	311.50	281.90
Total essential amino acid	87.83	85.11	93.70	56.60

From Li et al., 2006, 2009

is called “soft gold skin” (translation of a Chinese term). It can transport nutrients deep into the skin, nourish, moisten, repair, smooth and regenerate cells, ameliorate the inner environment, enhance immune functions, purify the blood, and stimulate blood circulation. The biological activities of sea cucumber collagen are as follows: (1) Antioxidant activity: it plays an important role in clearing away the excessive free radicals *in vivo*, and preventing membrane lipid peroxidation. (2) Antiaging effect: protective effect against aging induced by ultraviolet rays (mouse skin model). Synthetic sea cucumber collagen peptide can reduce MDA (malondialdehyde) content in serum and skin of mice exposed to ultraviolet light, increase hydroxyproline content (skin), and enhance SOD (superoxide dismutase) and GSH-px (glutathione peroxidase) activity (Cui, 2007). (3) It can prevent melanogenesis of B16 melanoma cell, and effectively reduce skin melanism, freckle, chloasma, and age pigment. (4) It has a protective effect for endothelial cells against oxidative damage, and plays an important role in antiatherosclerosis. (5) Antineoplastic activity: pentapeptide constituted by amino acid separated from sea cucumber epithelial tissue (some amino acids are D-type), relative molecular weight 568.1 Da has antineoplastic and anti-inflammatory activity. The synthetic product has the same effect (Fan, 2001). (6) Sea cucumber peptide has antifatigue effects (see also Chapter 13 for tissue biochemistry).

19.2 PHARMACEUTICAL PROPERTIES OF SEA CUCUMBER

19.2.1 HISTORICAL PERSPECTIVE IN TRADITIONAL CHINESE MEDICINE

Sea cucumber is known as “ginseng of the sea.” It is also called Liao sea cucumber (in *Medicine Guide* by Du Wenxie, Ming Dynasty), sea man (in *Wu Za Zu* by Xie Zhaozhe, Ming Dynasty), spiky sea cucumber, square beche-de-mer, square column sea cucumber, square sea cucumber, yellow meat, white beche-de-mer, and sea mouse. It has long been associated with many health benefits (see also Chapter 1). Keeping in mind that principles of traditional Chinese medicine are not easy to translate, even for native Chinese, we herewith present an overview of sea cucumber descriptions in ancient days.

Most documents refer to whole dried sea cucumber (beche-de-mer) of *A. japonicus*. The *Materia Medica of Food* by Yao Kecheng (Ming Dynasty) describes it as sweet flavored, salty, neutral, and nontoxic. Other documents use similar descriptors, including the *New Compilation of Materia Medica* by Wu Yiluo (Qing Dynasty) (sweet, salty, and neutral) and *Mai Yao Lian Zhu · Yao Xing Kao* by Long Bai (Qing Dynasty) (salty and cold). *The revised version of Materia Medica* by Ye Tianshi (Qing Dynasty) indicates that it is a good tonic for the heart and kidney, and that it is additionally good for the lungs, whereas the *Cui Jin Qiu Ben Cao Shu Lu* by Xu Lingtai (Qing Dynasty) and the *Summary of Materia Medica* by Chen Qirui (Qing Dynasty) both mention that sea cucumber is good for the meridian channels. Globally, several ancient books of records and efficacy (e.g., *Summary of Medicine Properties* by Wang Xunzhuan (Qing Dynasty); *Compendium Medical* by Wang Fu 1758; *Traditional Chinese Medicine* by Li Shizhen, 1590; *Modern Practical Traditional Chinese Medicine* by Ye Juquan 1956; *Chinese Pharmacology of Sea and Lakes* by Jia Yuhai 1996) agree that sea cucumber can nourish the kidney and strengthen the essence, nourish blood and promote the secretion of saliva, regulate menstruation and nourish the fetus, soothe dryness, favor hemostasis, dissolve phlegm, prevent eating disorders, facilitate urination, and kill mites that cause scabies. It is said to cure deficiency of the kidney, impotence, nocturnal emission, consumptive disease, deficiency of essence and blood, deficiency of yin, lung deficiency, chronic cough and cough caused by dryness, hemoptysis, aplastic anemia, postpartum weakness, neurasthenia, edema, constipation due to intestinal dryness, dysentery, hematochezia,

hemorrhoidal bleeding, and traumatic bleeding (reviewed by Wang, 1991). Ye Tianshi and Wu Jutong (Qing Dynasty), who were well-known doctors in the Qing Dynasty, believed sea cucumber was good for treating "warm diseases." They cured the condition known as Yang Ming disease with a decoction of *Glycyrrhiza uralensis*, *Panax ginseng*, *Angelica sinensis*, Chinese rhubarb, mirabilite, figwort, Liriope, and ginger to which sea cucumber was added. Some of the benefits of sea cucumber are not substantiated and are believed to be more folklore than truth; however, more and more research is demonstrating the advantages of incorporating sea cucumber into the human diet (see Section 19.2.2).

Apart from whole beche-de-mer, sea cucumber viscera, commonly known as sea cucumber intestines, were also perceived as a potent medicine. Chinese texts describe this product as "sweet, mild, and belonging to the stomach meridian" (Guan and Wang, 2009). It is said to relieve pain and convulsion, harmonize the stomach, and stop bleeding. It can therefore help treat epilepsy, indigestion in children, epigastric pain, gastric and duodenal ulcers, measles, sores and furuncles, and traumatic bleeding (Guan and Wang, 2009).

19.2.2 EFFICACY VALIDATION

Modern medicine has proved that there are various active substances in sea cucumber, such as mucopolysaccharide, Holothurian fucan (HF), glycosaminoglycan (HG), chondroitin sulfate, sea cucumber saponin, sea cucumber peptide and toxin, as well as nucleic acid, 18 kinds of amino acids, and more than 30 microelements, such as selenium, zinc, copper, manganese, vanadium, calcium, and phosphorus. Functions of these compounds include antithrombus, antitumor, and antipathogenic microorganisms, promoting wound healing, immunity enhancement, and antifatigue (Guan and Wang, 2009).

A. japonicus is considered the "king of sea cucumber"; its beche-de-mer extract boasts many properties including anticoagulation, antitumor, regulating blood-lipid, antivirus, analgesia, spasmolysis, and antiradiation. The acidic polysaccharide in beche-de-mer sea cucumber viscera also has antitumor and anticoagulation properties. Glucoside extracted from the gonad is effective in preventing radiation injury (Guan and Wang, 2009).

At present, sea cucumber, especially beche-de-mer, has been used in the treatment of aplastic anemia, disseminated intravascular coagulation, venous thrombus embolism disease, diabetes mellitus, fungal disease, phthisis, neurasthenia, impotence, stomach and duodenal ulcer, especially tumors, cardiovascular disease, immune disease, and senility diseases. Sea cucumber is also effective in treating postpartum rehabilitation, rehabilitation after illness, and some chronic diseases (Fan, 2001). There are now publications about the "Ginseng of the sea" in English literature, which demonstrates sea cucumber's excellent curative effect gradually being recognized around the world (Fan, 2001; Chen, 2003).

19.3 CONCLUSION

Sea cucumber is a rich source of >50 active substances, such as proteins, minerals, vitamins, polysaccharides, and lipids, which are of great nutritive value and exhibit medicinal properties. The body wall of sea cucumber (beche-de-mer) is the major organ used as food and as a source of active ingredients; its major constituents are collagen, acidic mucopolysaccharide, and saponin. Sea cucumber extracts have been proposed to allay fatigue, boost the immune system, strengthen resistance to disease, treat injuries, prevent inflammation, and provide liver and blood vessel protection. The active substance called holothurin has apparent inhibiting effects on various fungi. There are great amounts of holothurin (sea

cucumber saponin) in the sea cucumber body wall, viscera, and glands. Holothurin is also considered an antidote, nontoxic for humans, that can inhibit the growth and propagation of tumors. Overall, a number of active nutritive ingredients play varied roles in health care. Therefore, sea cucumber has been listed among the "eight famous sea foods" since ancient times, and it is deemed suitable for people of all genders and ages.

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CHAPTER

PROCESSING AND CUISINE 20

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SUMMARY

Sea cucumber, much like ginseng, is considered among the most nourishing foods in China. The traditional way of processing sea cucumber (i.e., dried body wall or beche-de-mer) has been passed along for more than 1000 years. The advantages of this preparation are its simplicity, low cost, stability (it keeps >10 years in storage), and ease of transportation. Other traditional methods include salted dried sea cucumber, salted fresh sea cucumber, unsalted fresh sea cucumber, and frozen dried sea cucumber. Modern processing methods include vacuum freeze drying, heat drying, microwave freeze drying, heat pump drying, instant sea cucumber, canned sea cucumber, and high-pressure processing, to name only a few. In this chapter, we outline and discuss the various processing methods and some of the most common recipes developed over the years around this exceptional seafood.

Keywords: *Apostichopus japonicus*; cuisine; processing; sea cucumber; seafood

20.1 INTRODUCTION

Sea cucumber is a prized seafood in China and other regions of Asia. It is attributed multiple dietary and health benefits (see Chapter 19), and as such it is one of eight Chinese delicacies alongside the well-known bird's nest, abalones, and shark fins. Since ancient times, ginseng (of terrestrial origin) and sea cucumber (of marine origin) have been almost equally well-known in China. The text *Wu Za Zu* (Five Assorted Offerings) by Zhaozhe Xie in the Ming Dynasty (1368–1644), states "The sea cucumber is a warm tonic with the same medical effect as ginseng, which is why it is called ginseng of the sea." Sea cucumber has always been regarded as a precious tonic, e.g., *Bencao Yixin*, a medical book written in the Qing Dynasty (1636–1912) as a supplement to the *Compendium of Materia Medica*, said sea cucumbers could "nourish kidney, enrich essence, strengthen *yang* (Traditional Chinese Medicine theory), and treat impotence." They also help prevent ageing of organs and skin, increase vascular tonus, control high blood pressure, and prevent coronary heart disease. Moreover, the consumption of sea cucumber has certain beneficial medicinal effects relative to the following diseases: kernicterus, edema, hepatitis, hemorrhoids, gastric ulcer, and even cancer. With their soft flesh, rich nutritive value, and delicate flavor, sea cucumbers are a typical high-protein low-fat food; they became a well-known delicacy in ancient Chinese palaces where they were often served as the last and finest dish at royal banquets in the grand hall. Their processing and cooking methods have been studied for more than a

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thousand years in China (see Chapters 1, 13, and 19 for details on biochemical composition and nutritional and medicinal properties).

20.2 HISTORY OF SEA CUCUMBERS AS EDIBLE FOOD

20.2.1 ANCIENT RECORDS

Chinese people first tasted sea cucumbers some 1800 years ago, in the era of the Three Kingdoms (220–280). Ying Shen, a scholar from Wuzhong, discussed how to eat sea cucumbers in his work entitled *Linhai Shuitu Yiwuzhi* (Treatise on the Anomalous Aquatic and Terrestrial Creatures of Linhai), which said, "The sea cucumber has black flesh and is 5 cm in length, as big as a baby's arm, with a mid-body abdomen and no mouth and eyes." In the era of the Han and Jin Dynasties (202–420), sea cucumbers were listed in some food books, such as Volume 6, *Yinshi Xuzhilu* (Dietary Notes) by Ming Jia, which stated, "With a sweet and salty flavor, sea cucumbers are cold and smooth. Patients with diarrhea may not eat sea cucumbers." From the Ming Dynasty onward, sea cucumbers became a delicacy in Chinese palaces. Bi Lv said in *Yinshi Haoshang of Ming Gong Shi* (Ancient Book Volumes of Delicate Diet, Court History of the Ming Dynasty), "The former emperor (Yuanzhang Zhu) liked eating sea cucumbers, abalones, and shark fins."

In the Qing Dynasty, there were more records of sea cucumbers as an edible food. Early on in that dynasty, Weiye Wu said in Volume 10 of his *Meicun Ji*, "Sea cucumbers originate from the Denglai Sea." Moreover, he wrote a famous text called *Sea Cucumbers*, which includes the following segment: "Wash sea cucumbers with well water firstly, and then put them into a pan for cooking. The forbidden area set for this royal tribute is as wide as the Beihai Sea, and this delicacy can be combined with southern dishes. No one can tell whether sea cucumbers are worms or fishes, or even whether they are plants. A person can eat sea cucumbers instead of medicines to promote health." In Volume 5 of the same series, *Lao Lao Heng Yan* (Health Manual for Elderly People), by Tingdong Cao indicated that sea cucumbers contributed to "kidney nourishing and *yin* invigorating"; *Linjielei of Suixiju Yinshipu* (Chinese Ancient Book), written by Shixiong Wang, ascribed similar properties to sea cucumbers, i.e., "kidney nourishing, blood enriching, *yang* strengthening, moistening, menstruation regulating and fetus nurturing"; in addition, in *Dongwulei of Qingbai* Leichao stated, "Sea cucumbers are echinoderms. In the past, they were called Sand Xun, and only the dry ones were called sea cucumbers. At present, they are generally called sea cucumbers; they live inshore and are edible after exposure to sun for drying."

20.2.2 ANCIENT CHINESE COOKING METHODS

Mei Yuan, a learned man in the south of Changjiang River, stated in his *Suiyuan Shidan* (a cooking book) that "Generally speaking, someone wishing to host a dinner tomorrow needs to soak and simmer sea cucumbers today for them to be thoroughly cooked…With expanded bodies, sufficient water absorption and soft flesh, the soaked sea cucumbers can be used for cooking various dishes. Once sea cucumbers have been rehydrated properly, they can be simmered in chicken soup and gravy after a few hours until well done." Mushrooms, such as *Auricularia auricula* and shiitake, *Lentinula edodes*, can be used as accompanying vegetables because they have a similar color. The sea cucumber cooking method described by Mei Yuan was commonly used in the Qing Dynasty, i.e., soaking with hot broth to remove the fishy smell and then using another kind of gravy to flavor the dish. Yizeng Ding, a

scholar from Rizhao during the Qing Dynasty, said in his work *Nongpu Bianlan* (Farm Handbook), "To cook sea cucumbers, immerse them in cold water to make them fully rehydrated at first. Afterwards, rub away their rough cuticles, clean and split them, remove their guts and cut them into slices. Boil them with salt water until well done, add thick gravy, transfer the mixture to a bowl, and then heat the bowl with hot water to make the mixture fully cooked. Wait for use." This processing method, which involved using water to soak as the first step and then heating again with gravy, was very popular in that period. In addition, ancient Chinese people tried many ways to cook sea cucumbers, which all achieved a satisfactory result. The following is a quote from Fuyun Wang's transcript of *Shixian Hongmi* by Yizun Zhu (1629–1709): "Boiling is a good way to eat sea cucumbers, which can also be eaten raw. It is also feasible to mix them with sauce or roast their flesh." Sea cucumbers do not have a naturally good taste. To produce an appealing dish, they must be cooked with flavored broth and/or sauce. The section on *Haixiandan* (Seafood Recipe) in Mei Yuan's *Suiyuan Shidan* (a cooking book) said, "Sea cucumbers are a product with various flavors, lots of sand and fishy smell, thus hard to cook. Due to their dense nature, they may not be soaked and simmered with pure water. To cook properly, pick small sea cucumbers, soak them in water to remove sand and mud, and then soak in hot broth three times."

Volume 6 of *Tiaodingji*, a famous cooking book written by an anonymous author during the Qing Dynasty, described four cooking methods, i.e., simmering, frying, braising, and stewing, each with its own benefits.

20.2.2.1 Simmering

Sea cucumbers themselves have a rather bland/mild or delicate taste, and simmering enables the meat to absorb flavors. In *Tiaodingji*, the method of simmering was described as follows: "Sea cucumbers are a suitable food in winter and in summer. Simmer them with pig's feet and add some spices." Mei Yuan also approved of this method and suggested "simmering until well done."

20.2.2.2 Frying

This is a relatively simple cooking method, which is used to prepare a dish in a short time. Yinshilei of *Qingbai Leichao* (Diets, Qing Petty Matters Anthology, the late Qing Dynasty) stated, "To make a fried dish use slices of sea cucumbers, fry and stew them with chicken, bamboo shoots and mushroom slices." Generally speaking, the frying method was used the least frequently to prepare a dish made of sea cucumbers in the past.

20.2.2.3 Braising

This is the most popular cooking method used to provide a delicious dish with meat or vegetables as complementary ingredients. *Tiaodingji* altogether recorded 11 dishes made using this method, which are listed as follows: braised sea cucumbers with agarics, braised sea cucumbers with puffer fish bellies, braised sea cucumbers with pig's tongues, braised sea cucumbers with pig's brains and agarics, braised slices of sea cucumbers, braised sea cucumbers with meat loaves of wild ducks, braised sea cucumbers with lotus and melon seeds, braised sea cucumbers with crab meat, braised sea cucumbers in the form of butterfly, braised sea cucumbers with pastries, and braised sea cucumbers with chicken livers and pork tenderloin.

20.2.2.4 Stewing

Volume 6 of *Tiaodingji* recorded stewed slices of sea cucumbers with clam slices or with pickled spinal tendons and hamstrings. Other historical records mentioned stewed sea cucumbers in oyster sauce. As

a unique style of cooking, stewed sea cucumbers has always occupied a leading position among sea cucumber dishes.

In the past, Chinese people cooked many different dishes with sea cucumbers. Other than the dishes just mentioned, there were also sea cucumber soup, gruel, and meatballs, among others. A semiliquid food, the thick soup was a favorite of Chinese people. *Haixiandan of Suiyuan Shidan* (1792) introduced the following cooking method: "Dice sea cucumbers and place them with diced bamboo shoots and mushrooms into chicken soup for stewing." Volume 6 of *Tiaodingji* described how to make sea cucumber soup with pheasant meat and emphasized the necessity of finely dicing and stewing until well done. Wen Fang, a poet in the Qing Dynasty, wrote a poem called Sea Cucumbers, which stated, "It is appropriate to make meat or vegetable soup with sea cucumbers." As a very beautiful dish, sea cucumber meatballs were made by cutting and shaping sea cucumbers into balls with stuffing made of ham, chicken skin, bamboo shoots, and then stewing those red and white balls to prepare the final product.

20.3 MODERN PROCESSING METHODS

Sea cucumbers can be marketed as fresh, dried, or deep-processed products. At present, the dried forms, including salted and unsalted dried sea cucumbers, are the primary products on the markets. With technical development, new processing methods are continuously being developed, such as vacuum freeze-drying, hot-air drying, microwave freeze-drying, heat-pump drying, cold-air drying, ready-to-eat fresh sea cucumbers, canned sea cucumber, high-pressure sea cucumbers, ultra-high-pressure sea cucumbers, and deep-processed foods (capsules, oral solutions, and enzymolysis products).

20.3.1 DRIED SEA CUCUMBERS

Fresh sea cucumbers are very fragile and dissolve into water (undergoing autolysis and liquefying) if not processed in a timely fashion after harvest. Once processed into dried products, sea cucumbers may resist mildew and worms (Lu, 2010) and be stored for a long time. They are the most common processed products and favored by numerous consumers. Based on processing techniques, the dried products are divided into the following four categories: salted dried sea cucumbers (Figure 20.1A), sugar dried sea cucumbers, unsalted dried sea cucumbers (Figure 20.1B), and frozen dried sea cucumbers (Figure 20.1C).

FIGURE 20.1

Dried sea cucumbers: (A) Salted dried sea cucumbers; (B) unsalted dried sea cucumbers; and (C) frozen dried sea cucumbers.

Photographs by Zhiling Li

20.3.1.1 Salted dried sea cucumbers

Live sea cucumbers destined for the production of salted dried products have to be more than 150 g wet weight, healthy, with lustered skin, and devoid of any ulceration. The main steps include the following: (1) Processing raw sea cucumbers – using a pair of scissors, an incision is made on 1/4 of the body length from the posterior ventral surface end (Figure 20.2A). The viscera (intestine, gonad, and respiratory tree) are removed from the body cavity (Figure 20.2B). The skin and body cavity are washed with fresh seawater – some companies use an automated cleaning production line for this operation (Figure 20.3A) – and then they are transferred into a barrel or basin. When the receptacle is full of eviscerated sea cucumbers, it is stirred with a wooden stick. This is a very important step. Through continuous stirring, the sea cucumber's tentacles will be retracted into the body cavity, and the processed sea cucumbers will not have their calcareous rings exposed at the surface of the body wall, which would negatively affect their appearance. (2) Boiling: The purpose of this procedure is to shape the soft raw sea cucumbers and eliminate some of the water content. First, seawater with a salinity of 30 is heated until boiling (Figure 20.3B, the automatic boiling line), and then the eviscerated sea cucumbers are added. When the sea cucumbers are just submerged and the water starts boiling, the preparation is left to simmer on a low fire. Foam should be scooped out and new water added as appropriate. This step is complete once the carcasses have stiffened and become slightly red. (3) Mixing with salt: the still warm and wet sea cucumbers are mixed with coarse salt (30–50% of total carcass weight). The mixture is stirred while cooling. (4) Salting: When the sea cucumbers are cold, they are placed into a porcelain jar or heat-insulated box with salt, and stored in a cool place for 10–15 days. (5) Roasting: This procedure is also called secondary boiling. The salted sea cucumbers are placed into a pot with seawater, heated on a strong fire until the water starts boiling, and then left to simmer at low intensity for 20–30 min, until the salt crystallizes. Then the sea cucumbers are removed from the water. (6) Blending with ash: A traditional processing procedure of salted dried sea cucumbers often includes addition of ash in order to protect them from moisture. Oak ash is generally preferred. After excess water has been removed following the second boiling, they are placed into a plastic container, mixed with oak ash (~10% of total

FIGURE 20.2

Processing raw sea cucumbers: (A) incision of the ventral surface with scissors; (B) viscera removal.

Photographs by Yuze Mao

FIGURE 20.3

Assembly line for sea cucumber processing: (A) automatic cleaning production line; (B) automatic boiling line; (C) low-temperature cold-air dryer chamber; (D) pure water production equipment.

Photographs by Zhiling Li

carcass weight) and their surfaces thoroughly coated. (7) Sun-drying: About 30 min after coating with oak ash, the sea cucumbers are distributed evenly on a cement platform for sun-drying until thoroughly dried (Figure 20.1A).

Through the method just described, 23 kg of fresh sea cucumbers, or about 11 kg of fresh eviscerated carcasses, is needed to obtain 1 kg of dried product. However, in order to reduce costs and pursue higher profits, many manufacturers often carry out a process called "scuffing" (mixing with a lot of salt) to increase the weight of dried sea cucumbers (a questionable procedure; see also Chapter 22).

20.3.1.2 Unsalted dried sea cucumbers

No-salt dry processing (Figure 20.1B) is a relatively new technique in which the steps of repetitive boiling and salting are eliminated. The nutritive value of sea cucumbers is optimally preserved, and the quality is more reliable (consistent). Hence, they are the consumers' favorite.

The processing method is as follows: (1) Processing of raw sea cucumbers and (2) boiling as described in Section 20.3.1.1. (3) Drying: Boiled sea cucumbers are left to cool down for 10 min, and then placed in a low-temperature drying zone (Figure 20.3C, the cold-air dryer chamber) at ~25–30 °C. After three or four days, when the sea cucumbers reach a moisture level of 40%, salt is added on the skin and rinsed away with freshwater. The sea cucumbers are then returned to the drying oven for another four or five days. Through this method, about 28–30 kg of fresh sea cucumbers, or 14–15 kg of fresh eviscerated carcasses, yield 1 kg of dried sea cucumber.

20.3.1.3 Freeze-dried sea cucumbers

The greatest advantage of freeze-dried sea cucumbers is that they are ready to eat after simple rehydration (Figure 20.1C). Moreover, they can be kept fresh without any additive or preservative (Yun, 2006). The whole processing procedure is carried out below the freezing point under vacuum (no oxygen), with minimal loss of bioactive components, and lower final moisture content. With their color, shape, and nutrients preserved, these sea cucumbers will have the same taste as fresh sea cucumbers some 6 h after rehydration at 25 °C. The main technical parameters of vacuum freeze-drying are as follows: freezing temperature of −25 °C; cold-trap temperature of −29 to −31 °C; vacuum at 10–20 Pa; final freeze-drying temperature of 60 °C. The surface and core of sea cucumbers processed in this manner will reach 60 °C after about 10 and 14 h, respectively, and will be ready after 18 h.

The steps in the traditional freeze-drying process are as follows. (1) Raw sea cucumbers are processed as per Section 20.3.1.1. (2) Scalding and shaping: Freshwater or pure water (Figure 20.3D, pure water production equipment) is brought to a boil, the processed sea cucumbers added and cooked on a strong fire until the water boils again. After 1–2 min, the sea cucumbers are removed and placed into icy freshwater and cut to remove the calcareous rings. (3) Freeze-drying: The preprocessed sea cucumbers are placed in a freeze-dryer at a temperature of −50 °C. The vacuum is turned on to sublime the ice into vapor, achieving the dehydration. This technique can preserve the color, flavor, and form of fresh sea cucumbers and their nutritional properties (Ke, 2010).

Yuan et al. (2010) improved the freeze-drying processing technique as follows. After removing the viscera from fresh sea cucumbers, they are boiled for 10 min and soaked for 48 h in purified freshwater at 4 °C. The standard procedure of quick-freezing is then used to prefreeze the sea cucumbers. Heating is initiated after the vacuum has been kept constant below 10 Pa for 0.5 h. The sea cucumbers are heated at 50 °C (ice sublimation for steam) for 3 h to sublime the ice, and the temperature increased to 70 °C until the sea cucumbers are thoroughly dried. With a plump appearance, erect tentacles and higher rehydration rate, this product is even better than the freeze-dried sea cucumber currently sold on the market (Yuan Wenpeng et al., 2010).

20.3.1.4 Other dried sea cucumbers

Additional techniques are used to dry sea cucumbers, including hot-air drying, heat-pump drying, cold-air drying, and microwave freeze-drying (Zhang et al., 2008; Zhu, 2012). Notably, changes to parietal collagen fibers during processing may be the main factor that determines tissue structure and quality of the processed products. When the temperature is higher than 100 °C, the primary structure of collagen is seriously damaged, with significant impacts on subsequent processing and preservation (Wang et al., 2011). The higher the temperature, the stronger the parietal water-absorbing capacity over the same period. For sea cucumbers, the limit of sea cucumber heat processing is 4 h at 100 °C (Dong, 2010).

20.3.2 FRESH SEA CUCUMBERS

20.3.2.1 Fresh sea cucumbers

Compared to traditional dried sea cucumber products, fresh sea cucumbers retain a higher degree of integrity and nutritive value. Moreover, the time-consuming rehydration step is eliminated when cooking them. However, they have a more pronounced fish smell and astringent taste, and their texture does not meet the taste requirements of being soft and glutinous. Hence, it is necessary to process them in one of two ways before consuming them: (1) The whole fresh sea cucumbers are briefly boiled and rinsed thoroughly with clean freshwater. The viscera are removed from an incision into the body wall (discussed earlier). Processed sea cucumbers are then placed into rice water, heated to a boil, and left to simmer for 4–5 min. They are then ready for cooking. This processing method is simple; however, the sea cucumbers prepared in this manner are less flavorful, and need some seasoning. They are suitable for making cold dishes, such as sea cucumber salad, boiled fresh sea cucumbers, wine-soaked sea cucumbers, etc. The rice wash water is used to soften the flesh of sea cucumbers and eliminate their fishy and astringent smell. The sea cucumbers are best left to cool down naturally rather than in cold water. (2) Fresh sea cucumbers are eviscerated, and stirred with egg white and white sugar for 10 min. They are then washed with clean water and placed into a pressure cooker with broth, onions, ginger, and aniseed, and cooked to a boil. After stewing for about 10 min, the sea cucumbers are taken out and immersed in hot soda water, where they are left to soak for about 4 h, before being thoroughly rinsed in clean water. They are then placed in clean water in a refrigerator for 5 h. After a final cleaning, they are boiled quickly one last time and ready to cook. The sea cucumbers processed through this method are suitable for making hot dishes, like braised sea cucumbers with spring onions, stuffed sea cucumbers, fried crispy fresh sea cucumbers, etc. The key factor is the boiling time in a pressure cooker. Fresh sea cucumbers should be processed quickly rather than stored for long periods (Jia, 2004).

Clean eviscerated fresh sea cucumbers can be sliced and dipped in sauce or mixed with vegetables after blanching. This process is simple and preserves all the original flavors and features of sea cucumbers. However, they are difficult to digest, and they may be associated with low absorption rate and poor taste when processed improperly. Braised recipes work well to enhance taste, soften sea cucumber tissues, and favor absorption of beneficial ingredients, such as amino acids and polysaccharides. However, heat-sensitive bioactive substances will be lost, thus reducing the nutritive benefits.

20.3.2.2 Ready-to-eat sea cucumbers

Packaged ready-to-eat sea cucumber products, also known as instant sea cucumbers, may provide convenience for consumers and help develop the sea cucumber industry. There are three processing techniques depending on the raw materials used.

The first technique uses live sea cucumbers as raw materials, and the following steps: (1) Residual sand and mud is washed away using seawater. Sea cucumbers are then transferred in clean and flowing freshwater below 10 °C to remove the remaining sediment. (2) The internal organs and the calcareous ring are removed. Incomplete removal of the calcareous ring will have an adverse effect on the final product's quality. Eviscerated sea cucumbers are washed thoroughly with cold freshwater and drained. (3) Salting below 10 °C for 90 min to increase flavor. (4) Sea cucumbers are then scalded for enzyme elimination and dehydration. Various procedures are used. The preferred way is twofold: first scalding salted sea cucumbers in hot water at 80–85 °C for 3 min, cooling, and then scalding a second time in boiling water at 95–100 °C for 4 min. (5) The sea cucumbers are subsequently placed in a sauce (or gravy) for flavoring (according to taste). The preferred formula is as follows: salt, 1.5%; cane sugar,

FIGURE 20.4

Common machinery for processing sea cucumbers: (A) vacuum packing machine; (B) spray-type fully automatic conditioning; (C) peptide hydrolysis tank.

Photographs by Zhiling Li

3%; chicken powder, 1.45%; monosodium glutamate, 0.45%; cooking wine, 2%. (6) The packaging material has an influence on both quality and appearance of the products (Figure 20.4A). Nylon, aluminum sesquioxide, or heat-resistant polypropylene should be selected for their insulation properties, meeting the requirements of high-temperature sterilization, and transparency (enabling the consumers to see the products). The air is vacuumed out and may be replaced with nitrogen (99.97%) to produce instant-sea-cucumber products with vacuum or nitrogen packs. (7) Products are sterilized using hot water spray (some large producers use fully automated equipment for this, Figure 20.4B), multistage heating, and two-stage cooling systems (Liu et al., 2008a).

The second process uses dried sea cucumbers as raw materials and includes soaking for expansion, removal of mouth and longitudinal muscles, processing with enzyme, enzyme elimination, soaking in clean freshwater, flavoring, dehydration, and vacuum packing. The optimal temperature for processing is 40 °C, processing time is 50 min, and soaking time 13 h at an enzyme concentration of 0.075%. Sea cucumbers processed under these conditions yield the best quality (Ye and Xie, 2008).

The third method uses frozen sea cucumbers as raw materials, and includes the following steps: preprocessing, draining, immersing in sauce, dehydration, vacuum packing, autoclave sterilization, and heat preservation. The processing involves stewing in water at 90 °C for 4 h (repeated three times). With regard to the processed final products, i.e., instant sea cucumbers (Figure 20.5A), their flesh has a good elasticity, beautiful color, and unique flavor, and the loss of nutrient ingredients is minimized (Tang et al., 2012).

20.3.2.3 Pressure-cooked sea cucumbers

Fresh sea cucumbers are used as raw materials to be processed into pressure-cooked sea cucumbers (Figure 20.5B). After preprocessing, they can be processed into diverse ready-to-eat sea cucumber products. At present, pressure-cooked sea cucumbers are mainly divided into two categories: one is frozen pressure-cooked sea cucumbers, and the other is canned (bagged) pressure-cooked sea cucumbers. They are preprocessed in the same way, but stored differently.

The main steps are as follows: (1) Processing of raw sea cucumbers as per Section 20.3.1.1(1). (2) Preprocessed sea cucumbers are placed into a pressure cooker and heated to 115–125 °C for 20–30 min. (3) Sea cucumbers are soaked in ice water for 24 h. (4) Soaked sea cucumbers are placed in a freezer (−20 °C) for individual freezing and then packaged. For canned pressure-cooked sea cucumbers,

FIGURE 20.5

Instant sea cucumbers: (A) canned sea cucumber; (B) pressure-cooked sea cucumber; (C) sea cucumber dietary supplement capsules.

Photographs by Yuze Mao

an assembly line filling machine is used, and an appropriate quantity of antibacterial peptide is added into the liquid to reach the concentration of 0.1% for protection against corrosion.

20.3.3 BY-PRODUCTS AND DEEP-PROCESSED PRODUCTS

20.3.3.1 Sea cucumber guts and flowers

Japan has a long history of marketing salted sea cucumber intestines (see Chapter 22); they are popular and listed as prized seafood. In order to promote comprehensive processing and utilization of sea cucumbers, meet the market requirements, and increase economic returns, the intestines of sea cucumbers disposed of as waste in the past are now processed into salted products, which have a significant economic value. The steps are as follows: (1) Fresh (live) sea cucumbers are immersed in a seawater tank >50 cm tall with a mesh screen (1 cm) at the bottom for about 3 h. Seawater is changed at least twice during this period. Sea cucumbers will evacuate sediment and the sand grains will sink to the bottom through the mesh. To avoid reingestion of sand grains, the distance from the screen to the bottom should be not less than 10 cm. Moreover, the top of the tank should be shaded against strong light, which may prevent the sea cucumbers from spreading evenly in the tank. (2) Once devoid of sediment the gut can be removed through an opening covering 1/3 of the body length. Residual feces are removed by pressing gently on the intestine, which is then cleaned in seawater. Whole intact intestines are thus obtained. (3) The cleaned intestines are subsequently rolled in refined salt to coat evenly, before being placed on a screen or strainer for 2 h. The intestines are rolled a second time on 10–15% refined salt to obtain a uniform coating. The products are stored in a small container. This procedure takes 1–2 days in May or June, or 5–6 days in late autumn. (4) The salted intestines are packaged in nontoxic plastic bags (0.5 kg). The air should be removed from the bags as much as possible before being sealed and stored at −15 °C. One ton of fresh sea cucumbers yields about 16 kg of sea cucumber intestines.

Sea cucumber flowers, also called sea cucumber ova, refer to the female gonads and are another commercial by-product of the production of beche-de-mer. The Japanese use them to make tea, wine, and soup, or produce a special sauce by salting and fermenting them into a very expensive product (see

Chapter 22 on Japanese sea cucumber foodways). At present, sea cucumber gonads are mainly produced by removing gonad tubules from the sexually mature individuals (May–June) and freezing them. Care is taken to avoid mixing the gonads with the respiratory trees and guts during processing (Ke, 2010).

20.3.3.2 Deep-processed sea cucumbers

As sea cucumber markets expand, consumers develop increasingly higher expectations. The products must have high nutritive value, be safe, consistent, and easy to prepare and consume. In recent years, various deep-processed products have been developed, including sea cucumber capsules and polypeptide solutions.

Sea cucumber capsules are mainly divided into two categories: sea cucumber gut capsules and common sea cucumber capsules (Figure 20.5C). Processing is similar for the two. (1) It is necessary to temporarily hold the live sea cucumbers in a clean flow-through seawater tank for more than one day, in order to enable them to discharge gut contents. (2) Sea cucumbers are then processed as per Section 20.3.1.1. (3) Processed sea cucumbers or sea cucumber intestines are placed in a freeze-dryer at −50 °C. (4) Materials are ground through a combined action of impact, cutting, and friction between movable and fixed disks. The ground materials will enter a collection bag automatically, and the powder will be filtered out. (5) The final step consists of automated capsule filling and compressing.

Liu et al. (2008b) suggested freezing and powdering sea cucumber products. In their opinion, this process has the advantage of preserving and balancing the active nutrients; moreover, producing ultrafine powder enables quick and appropriate absorption by the human body. It is unnecessary to use chemical reaction or enzymolysis, and no additive or preservative is added, in order to ensure the quality of this natural "green" food. It is possible to vacuum freeze and control particle size to improve the efficiency and reduce the time necessary to freeze-dry. Particles of 8 mm can undergo freeze-drying in 720 min, whereas it takes at least 2000 min to freeze-dry whole sea cucumbers, even processed ones (Liu et al., 2008b).

Sea cucumber polypeptides are generally called "liquid sea cucumbers." Biological enzymolysis is used to transform the essence of fresh sea cucumbers into a nutritive solution, using a sea cucumber peptide hydrolysis tank (Figure 20.4C). This technique not only breaks down the original macromolecule protein into absorbable micromolecules, but also improves the nutritional and medicinal effects of sea cucumbers by using enzymes to activate sea cucumber polysaccharides.

Modern biological experiments have proved that polypeptides have advantages compared to proteins and amino acids. They have the best absorption rate in the intestinal tracts, and some oligopeptides, like dipeptides and tripeptides, are absorbed more quickly than amino acids with the same composition; polypeptides have physiological functions, such as reduction of antigens, promotion of lipid metabolism, reduction of cholesterol, promotion of mineral substance absorption, antioxidation, etc.; some polypeptides can enhance human immunity and promote human metabolism; polypeptides have better acid and thermal stability and water solubility, and their viscosity changes slowly along with their concentration. Hence, polypeptides have been gradually accepted on the market and will likely become the most popular sea cucumber products (see also Chapters 13 and 19).

The process flow for "liquid sea cucumber" includes quick freezing of fresh sea cucumbers at −70 °C, slicing, grinding and gelation in a colloid grinder, addition of protease for hydrolysis, boiling for sterilization, addition of ethanol 95% to get a final ethanol fraction of 60%, stabilization for 12 h, centrifugation, collection of supernatant (mixture of sea cucumber polypeptides), purification by ultrafiltration, debitterizing and decoloring, and freeze-drying to obtain a powder of sea cucumber polypeptides.

20.4 COOKING SEA CUCUMBERS

20.4.1 PREPARATION BEFORE COOKING

Dried sea cucumbers need to be rehydrated before they can be consumed. Pure freshwater is the most appropriate liquid for the hydration of dried sea cucumbers; optimal results are obtained with an ice–water mixture at 0 °C. Removal of the longitudinal muscles (generally called tendons) may also improve the results (expansion size). Edible oil of animal or vegetal origin has no effect on the expansion and sensory quality of sea cucumbers.

Factory hydration starts with soaking dried sea cucumbers in tap water at room temperature for 1–2 days. The oral calcareous rings and longitudinal muscles are then removed. Sea cucumbers are heated in tap water until boiling, and left to simmer for 30–50 min (depending on the hardness of the sea cucumbers). After cooling, they are placed into a mixture of ice and water, in a refrigerator at 0–5 °C. After 24 h, the sea cucumbers are boiled in distilled water for 10–20 min, placed in ice–water after a period of cooling, and stored in the refrigerator at 0–5 °C. These steps are repeated until the sea cucumbers are fully hydrated (Liu et al., 2010). Home recipes involve immersion of dried sea cucumbers in pure freshwater for about 30 h until they get soft. They are subsequently cut open and scraped to remove the longitudinal muscles and inner membrane. Washed sea cucumbers are boiled in water for about 30 min depending on size and quality. After cooling, the sea cucumbers are placed in cool purified water for three days, with a daily change of water. The sea cucumbers are edible after this process (Zeng, 2011).

20.4.2 COMPLEMENTARY FOODS AND CONTRAINDICATIONS

Ducks and sea cucumbers are an appropriate match with the following features: invigorating the five *Qi* (vital energy in Traditional Chinese Medicine, i.e., Lung *Qi*, Heart *Qi*, Liver *Qi*, Spleen *Qi*, and Kidney *Qi*), nourishing the five organs (lung, heart, liver, spleen, and kidney), rehydrating the body, and quenching fever. Spring onions and sea cucumbers are another appropriate combination, with the following benefits: invigorating vital energy and tonifying the kidney, most suitable for pregnant and parturient women and elderly people. Tofu and sea cucumbers complement each other to produce the following: improving intelligence, promoting tissue regeneration and strengthening physical health, most suitable for pregnant and parturient women, elderly people, and children. Spinach and sea cucumbers are an appropriate match with the following features: enriching the blood, generating body fluid, and moistening dryness (viewpoint of *Traditional Chinese Medicine*). Bamboo shoots and sea cucumbers contribute to the following: nourishing *yin*, moisturizing lung, clearing heat, and nourishing the blood; most suitable for female, middle-aged, and elderly people (see also Chapter 19).

Unsuitable combinations with sea cucumbers include the following. Persimmons and sea cucumbers together may cause bellyache, nausea, and vomiting. Vinegar and sea cucumbers may cause the protein to coagulate and contract and negatively affect the taste. Guavas and sea cucumbers are also an inappropriate match, which may cause bellyache and nausea.

Various contraindications are reported in the literature with regard to sea cucumbers:

Bencao Qiuyuan (Search for the Origins of Materia Medica) (Qing Dynasty, time was not written down clearly): Patients with diarrhea or spermatorrhea should not eat sea cucumbers.
Bencao Yinshi Bian (Recognition of Materia Medica as Food, 1823*)*: Patients with diarrhea should not eat sea cucumbers.

Suixiju Yinshipu (Recipe of Suixiju Residence, 1861*)*: People with a weak and nonfunctional spleen, copious phlegm, diarrhea, or any exogenous pathogen should not eat sea cucumbers.
Bencao Xingchang (Materia Medica as Healthy Food) (unclear period): Sea cucumber causes heat stroke if consumed too liberally.
Bencao Haili (Advantages and Disadvantages of Materia Medica, Written in Qing Dynasty, published in 1982): People with blood disorders or typhoid should not eat sea cucumbers.
Yinshi Bencao (Food Materia Medica, 2009*)*: Never eat sea cucumbers with liquorice.

20.4.3 CLASSIC SEA CUCUMBER DISHES

Sea cucumber dishes are an important part of all eight major cuisine (i.e., Shandong, Sichuan, Guangdong, Fujian, Jiangsu, Zhejiang, Hunan, and Anhui cuisine) styles in China, except in Zhejiang Cuisine. They are even popular in Hunan, Sichuan, Anhui and other provinces that are distant from the ocean.

20.4.3.1 Braised sea cucumber with scallion

Cut sea cucumbers into small oblong pieces and reserve for use. Slice winter mushrooms. Wash and cut Peking scallions into pieces. Heat oil in a cooker, add the slices of mushrooms and bamboo shoots, and fry them until well done. Afterward, add the pieces of sea cucumbers and the mixed sauce (including oyster sauce, sugar, dark soy sauce, light soy sauce, and water), and boil the mixture until it gets thick. Add the pieces of Peking scallions, fry the dish until well done, and then serve it on a plate. This dish is a famous dish in the northern regions of China; it is fresh, soft, and smooth, with no residual gravy after eating. Benefits: Nutritive, lung nourishing, and kidney tonifying (Figure 20.6A) (Liu, 2010).

FIGURE 20.6

Classic sea cucumber dishes: (A) braised sea cucumbers with scallion; (B) healthy sea cucumbers with barley; (C) superior sea cucumber pot; (D) steamed sea cucumbers; (E) sea cucumber and oxtail soup; (F) braised sea cucumber with hasma; (G) supreme fresh sea cucumbers; (H) pressure-cooked sea cucumbers in sushi.

Photographs by Qian Liu

20.4.3.2 Healthy sea cucumbers with barley

Wash pearl barley and Thai fragrant rice with clean water, and use them with pure water to cook gruel. Afterward, mix the cooked gruel with a pot of thin soup, and add sauce for flavoring. Put sea cucumbers into the mixture, and boil until well done (Figure 20.6B) (Liu, 2010).

20.4.3.3 Superior sea cucumber pot

Prepare a pot of thin soup, and add sea cucumbers with *Pholiota nameko* (mushroom), *Dictyophora indusiata* (mushroom), pigeon eggs, and angelica. Boil for 2 min, and then add salt, monosodium glutamate, white sugar, chicken powder, chicken sauce, and lenthionine for flavoring. Add flowering cabbages and wolfberry fruits, and heat until boiling (Figure 20.6C) (Liu, 2010).

20.4.3.4 Steamed sea cucumbers

Mix crushed garlic with soy and oyster sauce to make gravy. Stir-fry minced pork belly and add salt, monosodium glutamate, and soybean sauce for flavoring. Reserve it. Wash sea cucumbers thoroughly, place in a bamboo steamer, and steam for 3 min. Remove from the steamer and place on a plate. Serve this dish with the crushed garlic, XO sauce™, gravy, and home-made chili sauce (Figure 20.6D) (Liu, 2010).

20.4.3.5 Sea cucumber and oxtail soup

Soak and wash sea cucumbers. Cut fresh oxtails into slices, soak in water for a while, and then use them to make oxtail soup. Boil flowering cabbages in water briefly and reserve for later use. Cut open sea cucumbers longitudinally and clean their guts. Boil them in salty water for 20 min, and then boil in freshwater for 20 min. Soak them in ice water and take out after 12 h. Afterward, immerse them in the oxtail soup for stewing. When the oxtails are cooked thoroughly, add garlic slices, wolfberry fruits, red dates and flowering cabbages and stew for a while. Add salt, monosodium glutamate, and cooking wine for flavoring. Take the mixture out of the pot and scatter chives over it to complete the dish (Figure 20.6E) (Liu, 2010).

20.4.3.6 Braised sea cucumber with hasma

Stew the hydrated sea cucumbers in thin soup until well done. Remove and place in a container for later use. Prepare another pot of soup and heat until boiling. Add hasma and stew until well done. Remove and place in the container with the sea cucumbers for later use. Add salt and cooking wine into the abalone sauce and mix well. Heat a casserole and add chicken fat. Pour the mixed abalone sauce into the casserole and heat until boiling. Afterward, pour the sauce into the container with the sea cucumbers and hasma. The dish is now ready to be served (Figure 20.6F) (Liu, 2010).

20.4.3.7 Supreme fresh sea cucumbers

Cut open fresh sea cucumbers longitudinally and wash them thoroughly. Cool them with ice cubes for 20 min to produce the sea cucumber slices (their size should be twice their original size). Afterward, soak those sea cucumber slices in rice wash water, and heat them in boiling water for 1 min. Take them out and place on a plate with flowering cabbages that have been soaked briefly. Boil a pot of thin soup. After flavoring, thicken the soup and pour it on the sea cucumbers to finish the process. This dish offers a more delicious taste if eaten with crushed garlic (Figure 20.6G) (Liu, 2010).

20.4.3.8 Pressure-cooked sea cucumbers in sushi

Cook fresh sea cucumbers in a pressure cooker for 20 min, and then soak them in ice water for further use. Wash Thai fragrant rice and place in a steamer for steaming. Add horseradish, salt, and chicken sauce for flavoring. Reserve the rice for further use. Lay lavers on a sushi mat, put taxus leaves on it, and then add the processed sea cucumbers and fragrant rice. Roll the sushi mat tightly, and cut the sushi into rhombic pieces (Figure 20.6H) (Liu, 2010).

20.4.3.9 Raw sea cucumber

Both ends of the sea cucumber are first cut off (Figure 20.7A). Then, remove the sea cucumber intestines and the mucilage glue by adding some salt; salt is removed by washing. The body wall is cut into slices (Figure 20.7B, C). The green onion is cut into slivers. The body wall of the sea cucumber, red pepper, garlic soy sauce, and green onions are mixed together (Figure 20.7D).

FIGURE 20.7

Raw sea cucumber preparation: (A) removal of anterior and rear ends; (B–C) thin slicing of body wall; (D) final sashimi plate with sauce and vegetables.

Photographs by Qiang Xu

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CHAPTER

Apostichopus japonicus IN THE WORLDWIDE PRODUCTION AND TRADE OF SEA CUCUMBERS

21

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SUMMARY

Sea cucumber resources occur in oceans all over the world. Among them, one of the most common temperate species is the sea cucumber *Apostichopus japonicus*, which has been cultured successfully in China. The international trade structure for sea cucumber is different from that of typical marine products. About 90% of trade volume goes through Hong Kong and Singapore, while the Chinese mainland is the main consumer market. In most countries, sea cucumber is mainly exported in its dried form, whereas fresh, salted, and frozen products only take up a small part of the international trade. In this chapter, the production, import and export trends, and the main markets for all commercial species of sea cucumbers will be discussed. Emphasis will be placed on the local trade of *A. japonicus* in China, Japan, and Democratic People's Republic of Korea and Republic of Korea, and on market incentives and developments in China.

Keywords: *Apostichopus japonicus*; export; import; market; production; sea cucumber; trade

21.1 INTRODUCTION

Sea cucumbers are found in seas and oceans worldwide, from the intertidal zone to the deep sea. Harvesting and trading of sea cucumbers in Asia date back more than 1000 years. The Chinese consumer market is the main driving force behind sea cucumber fishery and aquaculture production in China and in most places around the globe. Wild sea cucumber resources are facing great threats, including overfishing, increasing human population, and loss of habitats (Purcell et al., 2011, 2014; Bruckner, 2001).

Wild sea cucumbers in tropical areas are captured by hand or hand tools, such as a spear and a hook. Harvesters may walk on the reefs, use a snorkel, scuba, or hookah (air compressor). Trawl fisheries are chiefly used by industrialized countries in temperate cold areas, such as Canada and Russia (Hamel and Mercier, 2008). This technique is only appropriate for sea cucumbers with high biomasses and widespread distributions that typically have comparatively low market values (Aumeeruddy and Payet, 2004). Another two species *Parastichopus nigripunctatus* ("okiko") and *Cucumaria japonica* ("kinko") are commercially harvested in small volumes in Japan (Akamine, 2004; Hamel and Mercier, 2008). Commercial sea cucumber populations in most areas are undergoing severe declines, which are

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compounded by the rapid development of the fisheries and may lead to population crashes (Uthicke and Benzie, 2000). Therefore, wild sea cucumber resources face great pressures in most places around the world. Stocks of the most prized species are already quite low; some are even listed on the IUCN Red List of threatened species, including *Apostichopus japonicus*, which is categorized as endangered (Hamel and Mercier, 2013), and will likely take decades to recover (D'Silva et al., 2001; Uthicke, 2004; Ahmed, 2007; Purcell et al., 2014).

The main sea cucumber species grown in aquaculture are *A. japonicus* (temperate species), *Isostichopus fuscus* (tropical species), and *Holothuria scabra* (tropical species). While *H. scabra* is mainly cultured in the Indo-Pacific and *I. fuscus* on the western coast of Mexico, both at small scales, the aquaculture of *A. japonicus* has developed into a large-scale industry in countries along the western North Pacific Ocean (particularly in China; see also Chapters 16 and 17). In the 1950s, Chinese scientists carried out research on artificial breeding and aquaculture technology of *A. japonicus* (see Chapters 7 and 8). Japanese researchers had already started working with this species as early as 1901 to answer questions related to their reproductive cycle as well as artificial breeding. There are now mature technologies for large-scale production of *A. japonicus* in China, Japan, and both Koreas. China is the major producer of *A. japonicus*; however, Japan also produces important quantities of *A. japonicus*, most of which are released for enhancement of natural populations.

The international trade structure for sea cucumbers is different from typical aquatic trades. The demand is limited to Chinese and other Asian consumers (often of Chinese origin), and the trade is also controlled by these groups. About 90% of the trade volume goes through Hong Kong and Singapore, while the Chinese mainland is the main consumer market (see Chapter 22). Sea cucumber is chiefly exported in dried form (beche-de-mer), whereas fresh and frozen products only take up a small proportion of the international trade. Although a substantial amount of data exists on the world sea cucumber trade and management, collated by the Food and Agriculture Organization (FAO), there is a call for more information on export and import volumes. In particular, a very small portion of the available information relates to *A. japonicus*, despite the fact that it is the most widely consumed sea cucumber in China. Indeed, the trade situation of *A. japonicus* remains relatively obscure (Ferdouse, 2004) because its production in China is mainly aimed at local markets. This chapter hopes to fill the knowledge gap by providing an overview of the sea cucumber resources, trade, and markets, with a focus on *A. japonicus*.

21.2 SEA CUCUMBER FISHERY RESOURCES

There are about 1200 species of sea cucumbers, among which about 70 species are commercially exploited, including temperate and tropical species (Purcell et al., 2012).

21.2.1 TEMPERATE RESOURCES

Temperate fisheries are divided into the Western Pacific, Eastern Pacific, and North Atlantic regions.

The Western Pacific region, where *A. japonicus* is the main commercial species, centers on the Chinese, Japanese, and Korean fisheries. *A. japonicus* has been bred artificially, and there are many large-scale aquaculture facilities in all three countries (especially in China). This region also covers the Russian fishery, but very limited information about Russian sea cucumber resources and exportations are available. There is also a growing New Zealand fishery focused on *Stichopus mollis*, which is presently an "exploratory fishery" under New Zealand's quota management system (Stenton-Dozey and Heath, 2009).

In the Eastern Pacific, small but growing fisheries are occurring in Canada (British Columbia) and the United States (Alaska down to California) (Conand, 2001). These temperate fisheries are based essentially on *Parastichopus californicus*.

North Atlantic fisheries are centered on *Cucumaria frondosa*, which is fished in Canada and the United States. *C. frondosa* is the most common species of sea cucumber in the North Atlantic ocean (Nelson et al., 2012; Christian et al., 2010) and one of the most abundant in the world (Hamel and Mercier, 2008). Its distribution ranges from the Arctic to Cape Cod (USA) and along Iceland and Greenland, down the coast of northern Europe and Scandinavia as well as the Faroe Islands (Singh et al., 1998; Hamel and Mercier, 2008).

21.2.2 TROPICAL RESOURCES

Sea cucumber resources in tropical regions of the Indo-Pacific and America usually comprise a diversity of commercial species. There are several dozen tropical species exploited commercially, which can be divided into three categories according to abundance, size, thickness, and quality of the body wall, main market demand, and value. The first category (comprising the most valuable tropical species) includes, among others, the brown sea cucumber *I. fuscus*, the white teat fish *H. fuscogilva*, the golden sandfish *Holothuria lessoni*, and the sandfish *H. scabra*. The second category (medium market value) includes the brown fish *Actinopyga echinites*, the black fish *Actinopyga miliaris*, and the prickly red fish *Thelenota ananas*. The third category (low market value) includes *H. atra*, *H. fuscopunctata*, *Stichopus chloronotus*, and *S. variegatus* (Conand and Bryne, 1993; Purcell et al., 2014).

21.3 MARKET TRADE

21.3.1 MAJOR EXPORTING COUNTRIES AND REGIONS

21.3.1.1 Temperate regions

As mentioned earlier, the major sources of temperate sea cucumbers are China, Japan, Democratic People's Republic of Korea, Republic of Korea, Canada, Iceland, USA, and Russia. Among these, the main exporting regions include Hokkaido for Japan, Maine for USA, and the Atlantic Provinces for Canada (Bruckner, 2001; Nelson et al., 2012).

Depending on the conversion factor used for the dry/wet weight of sea cucumbers, the combined catches for the Asian and Pacific regions are in the order of 20,000 to 40,000 t per year (Toral-Granda et al., 2008). The temperate areas of the Northern Hemisphere are also responsible for a substantial (and steadily growing) share of the world catches (in the order of 9000 t yr^{-1}); catches being sustained mostly by *Cucumaria frondosa*. Sea cucumber catches are less important in Africa and in the Indian Ocean (2,000–25,000 t yr^{-1}) and particularly low in Latin America and the Caribbean regions (<1,000 t yr^{-1}) (Toral-Granda et al., 2008).

Besides the catches of wild populations, temperate aquaculture (e.g., for *A. japonicus*) satisfies a substantial portion of the Chinese market needs. China owns the largest aquaculture industry of *A. japonicus* and successfully produces an estimated over 100,000 t wet weight of *A. japonicus* each year mainly to sustain domestic demand (Ministry of Agriculture China, 2009–2012). This value may be in the same order of magnitude (possibly even more) as the total world wild catches.

21.3.1.2 Tropical regions

In Southeast Asia, important sources of tropical sea cucumber include Indonesia, the Philippines, Vietnam, Thailand, and Malaysia (Perez and Brown, 2012). Although Indonesia is the largest producer of tropical sea cucumbers, the statistical data are incomplete in FAO records. The annual export was generally above 4000 t (dry weight) from 1987 to 1990, with values that reached 4888 t in 1989 (Anon, 2002, Tuwo, 2004). In 2000, Indonesia exported over 2500 t of sea cucumbers to the global market. Nearly 50% of these went to Hong Kong, followed by Singapore, China, Taiwan, Republic of Korea, and Malaysia; a small quantity went to Japan (Ferdouse, 2004).

In the Pacific Ocean region, the main suppliers of dried sea cucumbers for Asian markets are Papua New Guinea, Solomon Islands, Fiji, and Australia. However, the export volume of sea cucumbers in the mentioned Pacific Island countries showed a declining trend because local governments have adopted regulations to protect their sea cucumber resources (Ferdouse, 2004).

Recently, sea cucumbers from the Middle East (i.e., United Arab Emirates and Yemen) and Africa (i.e., Mozambique, Kenya, Madagascar, and South Africa) have entered the Asian market, among which 98% are in dried form. In the Indian Ocean, the main producers are Sri Lanka, Maldives, and India. Compared to Southeast Asia and the Pacific Ocean regions, this region supplies a small percentage of the global catches of sea cucumbers. Exports for main regions are listed in Table 21.1 (Fisheries and aquaculture software, 2013) (Figure 21.1).

21.3.2 MAJOR IMPORTING COUNTRIES

Global sea cucumber importations amounted to nearly 7500 t in 2008 (Fisheries and aquaculture software, 2013). The top five importing countries were Hong Kong (China), mainland China, Taiwan, Republic of Korea, and Singapore. Imports by these countries increased by 15%, reaching 8613 t in 2010. The estimated value peaked at 365 million dollars, corresponding to a sixfold increase over the last decade. Sea cucumbers are usually sold dried, but the demand for frozen sea cucumbers from mainland China and Hong Kong is growing. Imports for main regions are listed in Table 21.2 (Fisheries and aquaculture software, 2013) (Figure 21.2).

21.3.3 MAIN TRADE MARKETS

21.3.3.1 Mainland China

China is the largest consumer market of sea cucumbers. The temperate sea cucumber *A. japonicus* has become a very important aquaculture species in China, destined mainly to domestic markets. Due to the important variability in the purchasing power of consumers in mainland China, the Chinese market is able to absorb many types of sea cucumber products. Furthermore, markets in mainland China and Hong Kong are closely tied. Most of the dried sea cucumber imported by Hong Kong is reexported to Guangzhou, in Guangdong province, from where they are traded throughout China (Akamine, 2012).

21.3.3.2 Hong Kong

Hong Kong is the world's largest market for dried sea cucumbers, and is also the largest importer internationally. Between 2000 and 2009, it was handling an average of 54% of the global imports (Akamine, 2012). Hong Kong mainly imports tropical dried sea cucumbers including *H. scabra*, *H. fuscogilva*, *Thelenota ananas*, *H. whitmaei*, and *Actinopyga lecanora*, which come from Indonesia, Philippines, and Pacific Island countries; among them, Indonesia contributes about 40% (Perez and

Table 21.1 World Exports of Sea Cucumber (All Species), Fresh/Frozen/Dried/Salted, 2002–2011

Country/Region		2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
China (mainland)	Q	581	152	36	76	186	275	115	75	107	715
(Other than live, fresh, or chilled)	V	519	244	350	393	1,006	2,313	2,446	1,567	8,376	43,196
Hong Kong (reexportation)	Q	2,944	2,529	4,074	3,989	3,564	4,149	3,922	4,358	5,457	5,699
	V	16,676	13,518	28,480	25,847	24,459	31,041	31,917	28,734	34,539	36,302
Singapore	Q	867	1,134	1,231	942	515	363	426	271	236	219
	V	16,568	19,430	19,641	18,604	13,432	13,403	15,223	11,706	12,638	13,209
Maldives	Q	191	239	182	118	88	113	84	53	209	322
	V	2,972	3,371	2,426	1812	993	855	616	302	593	919
Thailand	Q	102	121	112	97	93	93	64	114	91	22
	V	298	285	156	182	138	200	48	48	—	—
Philippines	Q	1,407	1,009	1,079	—	—	—	—	—	—	—
	V	4,420	3,264	4,928	—	—	—	—	—	—	—
Solomon Islands	Q	253	376	48	269	—	—	—	—	—	—
	V	853	393	253	1,749	—	—	—	—	—	—
Sri Lanka	Q	213	104	—	96	—	—	—	—	—	—
	V	4,260	2,547	—	1,936	—	—	—	—	—	—

Q *represents the quantity in metric tons;* V *is the value × 1000 US$.*
From FishStat software, 2013

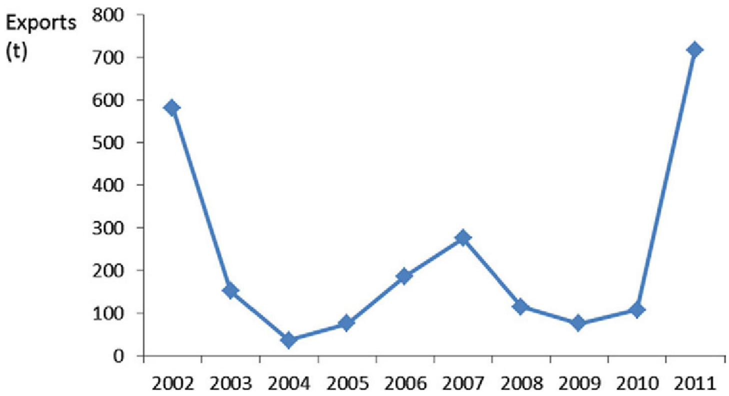

FIGURE 21.1

Sea cucumber exports (all species, other than live, fresh, or chilled) over time in mainland China.

From FishStat software, 2013

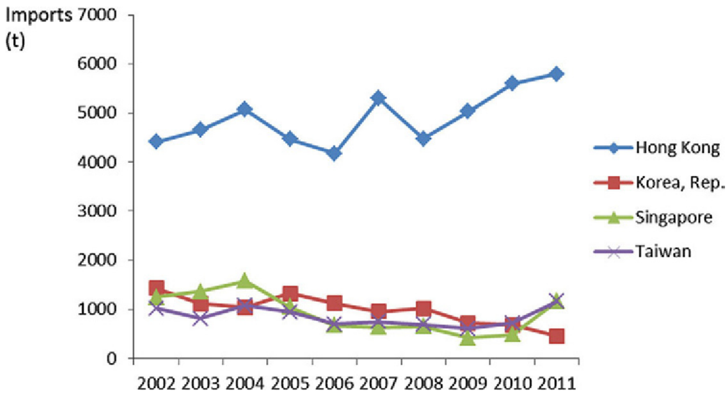

FIGURE 21.2

Sea cucumber (all species) imports over time in the major importing countries.

From FishStat software, 2013

Brown, 2012). Hong Kong also deals with temperate species of sea cucumbers from North America, Northern Europe, and New Zealand, chiefly in the form of dried and/or frozen body wall. In addition, Hong Kong imports the sea cucumber *A. japonicus* from aquaculture facilities in the Shandong and Liaoning provinces of China.

In 2007, Hong Kong imported 5296 t of dried sea cucumber: Papua New Guinea exported the most to Hong Kong (704 t of dried sea cucumber), Indonesia (653 t) second, and Japan (585 t) third (Akamine, 2012). Of the total dried beche-de-mer imported into Hong Kong, 83.5% was immediately or subsequently reexported (Akamine, 2012). According to the monthly statistics of Hong Kong, it reexported 4149 t of dried sea cucumber to 13 countries and regions in 2007. Among them, China imported 3576 t (86% of the total reexport volume from Hong Kong).

In Hong Kong, the major sea cucumber buyers are wholesalers and dealers of dried sea cucumbers, as well as high-end Chinese restaurants and supermarkets. The market for frozen cold-water sea cucumbers is expanding, mainly supplying specialized restaurants in Hong Kong and the southern provinces of mainland China.

21.3.3.3 Singapore

Singapore is the second most important trade center of dried sea cucumbers (Ferdouse, 2004). Major suppliers include Indonesia, Hong Kong, Maldives, Tanzania, and South Pacific island countries. Among them, Hong Kong is the main supplier. Indonesia also exports large amounts of sea cucumbers to Singapore, but these data are not reflected in the official statistics. Imports fluctuated from 820 t in 1997 to 629 t in 2000. Since 2005, the import volume of Singapore has declined, down to 423 t in 2009 (Table 21.2).

Domestic consumption of sea cucumbers in Singapore has been low over the last few years. The lion's share gets reexported to Hong Kong, Malaysia, Taiwan, and Thailand. The ASEAN Free Trade Agreement also allows reexports to member countries (Malaysia, Thailand, Myanmar) (Ferdouse, 2004).

Table 21.2 World Imports of Sea Cucumber (All Species), Fresh/Frozen/Dried/Salted, 2002–2011

Country/Region		2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
China (mainland)	Q	481	1,619	1,630	281	485	789	588	470	1,053	2,255
(Other than live, fresh or chilled)	V	1,282	3,459	4,603	2,249	3,276	7,389	5,363	3,995	11,067	5,987
Hong Kong	Q	4,417	4,655	5,070	4,474	4,180	5,296	4,477	5,037	5,600	5,798
	V	56,145	77,324	99,841	111,770	164,230	232,487	202,091	228,176	302,751	370,243
Korea, Rep.	Q	1,429	1,116	1,040	1,328	1,131	957	1,027	720	681	461
	V	5,518	4,695	6,122	8,208	9,051	12,257	11,240	8,795	12,919	14,924
Singapore	Q	1,253	1,364	1,578	1,053	672	637	653	423	491	1,160
	V	18,526	19,597	24,283	21,133	15,077	18,895	19,293	16,207	18,384	22,319
Taiwan	Q	1,019	818	1,082	946	706	747	698	611	725	1,162
	V	4,292	4,867	8,721	10,034	7,098	10,235	9,289	9,662	10,600	17,423
Thailand	Q	116	108	67	70	46	38	22	21	22	20
	V	472	387	178	168	126	99	103	83	235	154
Malaysia	Q	184	186	95	211	213	310	257	154	181	211
	V			203	440	428	695	683	786	810	1,005
Japan	Q	1	—	5	1	2	6	1	—	5	1
	V	36	54	71	49	124	213	78	19	155	22

Q *represents the quantity in metric tons;* V *is the value × 1000 US$.*
From FishStat software, 2013

21.3.3.4 Malaysia

Malaysia is a multiethnic country, where people of Chinese descent make up 30% of the total population. Over the past 10 years, the Malay population has been continuously growing, whereas the number of Chinese immigrants declined, which led to a contraction of sea cucumber markets. Other ethnicities in Malaysia, such as Malaysian and Indian, do not like sea cucumbers; moreover, consumption of sea cucumbers by Chinese immigrants in Malaysia is not as high as that in Singapore and Hong Kong. Malaysia imports a few fresh and frozen sea cucumbers, but the dried form constitutes the main market product. Almost half of the sea cucumbers imported by Malaysia are reexported to other countries. The import volume decreased from 536 t in 1999 to 154 t in 2009, and the main supplier is Indonesia (Table 21.2).

21.3.3.5 Other markets

The sea cucumber consumption in Japan, Republic of Korea, and Southeast Asia countries is not high. In Japan, sea cucumbers are mainly served in Chinese restaurants and the import volume of frozen and dried sea cucumbers is a mere 3–4 t yr^{-1}. Actually, the export volume of sea cucumbers is far higher than the import volume in Japan. Sea cucumbers in Japan are mainly exported to Hong Kong and

mainland China (see also Chapter 22). The import volume of sea cucumbers in Republic of Korea was 717 t in 2009, a decrease of 28% compared to 2008. The main suppliers are Indonesia, the Philippines, Japan, and Hong Kong (Table 21.1, Table 21.2).

In Vietnam, Indonesia, Thailand, and the Philippines, sea cucumbers are consumed by people of Chinese origin. As for occidental markets, such as the United States, Canada, United Kingdom, France, and Belgium, they mainly cater to Chinese immigrants, especially to elder people, and therefore sea cucumber consumption is decreasing gradually in these countries.

21.4 SEA CUCUMBER MARKETS IN CHINA

Aquaculture is an important segment of the Chinese sea cucumber industry. In the 1950s, China carried out research on the artificial breeding of *A. japonicus* for aquaculture. In the 1970s, it began to transfer sea cucumber seedlings back into the sea for stock enhancement, and in the 1980s, China started developing large-scale facilities for artificial breeding of *A. japonicus* in ponds (see Chapters 7, 8, and 16). Over the 1990s, the polyculture technology was developed (see Chapter 17), and aquaculture techniques in general were refined. A better understanding of aestivation (see Chapter 11) has promoted the development of industrial aquaculture of *A. japonicus*. Up to now, the main breeding techniques include artificial pond culture (in shrimp ponds and reef lagoons in the intertidal zone), purse-net culture in shallow seas, cofferdam breeding, sea-chest (sea-cage) culture, marine raft culture, and industrial aquaculture (Chang et al., 2006) (see Chapter 16). With the continuous improvement of seed production and breeding technology, sea cucumber culture has boomed in the northern coastal region of China, and the production output has increased rapidly (Chang et al., 2006).

21.4.1 RESOURCE DISTRIBUTION

China is a large consumer of sea cucumbers for cultural, health, and medicinal reasons (see Chapters 1 and 19).

There is only one edible species of sea cucumber native to northern China, which is *A. japonicus*; it is distributed in the shallow waters along the coasts of Liaoning, Hebei, Shangdong, and around Pingshan Island off the Lian Yunguang City in Jiangsu in the south. Among these areas, *A. japonicus* is most widely distributed along the coasts of Dalian and Jingzhou in Liaoning Province, as well as Yantai and Qingdao in Shandong Province (see Chapter 3). There are over 10 species of commercial sea cucumbers in southern China, among which *Stichopus variegatus* is the most commonly consumed. These sea cucumbers usually colonize rocky intertidal areas or coral reefs; they are found in the coastal waters of Hainan, Leizhou Peninsula, and Xisha Islands (Zhang et al., 2004).

The Chinese advocated the use of sea cucumbers as a folk remedy in ancient times (see Chapter 19). Following a growing focus on health care, sea cucumbers are becoming a popular tonic food in China. While dried products still dominate the Chinese markets, various sea cucumber products have recently appeared, ranging from oral liquid to dry pills (see Chapter 20). Stimulated by the prospect of large profits, capital investment has been continuously flowing into the farming and processing industries. The rational development of, and further research into, sea cucumber production should hence be thoughtfully considered by relevant authorities, investors, and farmers, as well as academic institutes (Chen, 2004).

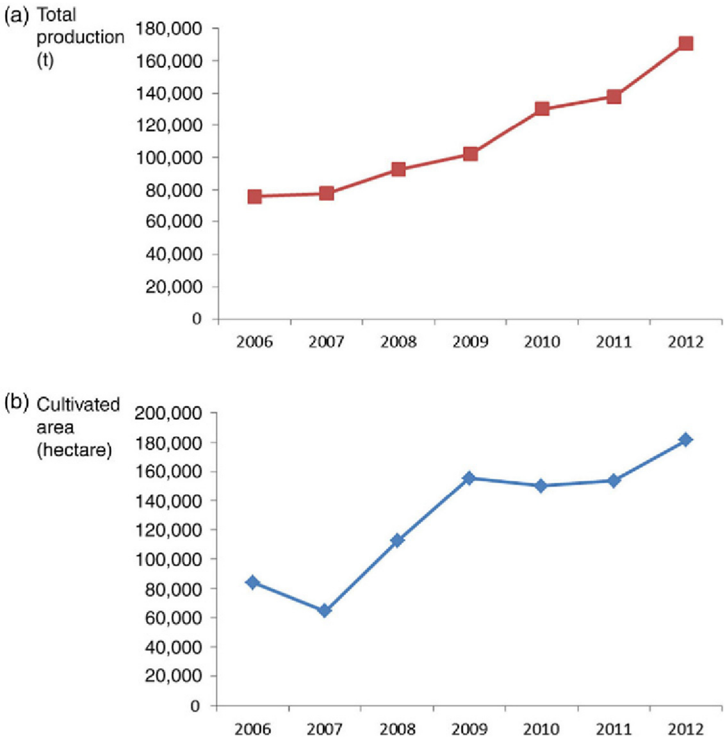

FIGURE 21.3

(a) Total production (wet weight) and (b) cultivated area for *A. japonicus* in China.

From Ministry of Agriculture China, 2006-2012

21.4.2 SEA CUCUMBER OUTPUT

21.4.2.1 Sea cucumber output and breeding area

In 2006, aquaculture production of sea cucumbers in China was 75,725 t, from culture areas that covered some 84,200 ha. Compared to that of 2005, the output and breeding areas had increased by 15.99% and 13.80%, respectively. In 2007, the sea cucumber production increased to 77,517 t, and the breeding area was 64,386 ha. In 2008, the output leaped to 92,567 t, and the breeding area to 112,468 ha. In 2009, the output and the aquaculture areas reached 102,159 t and 155,288 ha, respectively. In 2010, the output of cultured sea cucumbers was up again to 130,303 t and the aquaculture areas reached 150,113 ha. In 2011, the output and aquaculture areas were steady compared with those in 2010, with 137,754 t and 153,626 ha, respectively. The total production in 2012 increased to 170,830 t and cultivated area leaped to 181,514 ha (Figures 21.3 and 21.4).

21.4.2.2 Industrial pattern

According to statistics in China in 2006 (Ministry of Agriculture China, 2006), the output of Shandong and Liaoning provinces took up 98% of the total production of 75,725 t. Shandong produced 53,080 t of *A. japonicus*, accounting for 70.10%, and Liaoning produced 21,236 t, accounting for 28.04%. The

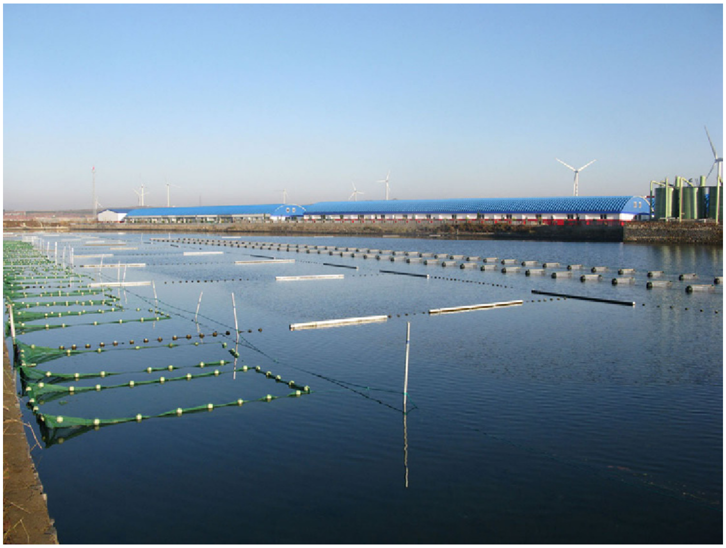

FIGURE 21.4

A pond where *A. japonicus* is cultured in Weihai, Shandong, PR China.

Photo by Shilin Liu

output of Hebei, Fujiang, and Jiangsu were 1064, 324, and 21 t, respectively. The southern provinces took advantage of large sea cucumber yield per unit. The yield per unit in Fujian Province could be up to 202,520 kg ha^{-1}, which was higher than 2135, 2100, and 1317 kg ha^{-1} in Shandong, Jiangsu, and Hebei provinces, respectively.

In 2007, the national total *A. japonicus* output was 77,517 t (Ministry of Agriculture China, 2007). Shandong ranked first with 53,573 t, and Liaoning second with 22,158 t. The sea cucumber output of Fujiang, Guangdong, and Hebei provinces were 651, 549, and 515 t, respectively. As far as the aquaculture areas were concerned, Liaoning and Shandong dominated, with 34,688 and 28,848 ha, respectively. Although the culture areas were small in Guangdong and Fujian provinces (22 and 5 ha, respectively), the yields were higher than in other areas, i.e., 109,800 and 29,591 kg ha^{-1}, respectively.

In 2008, the national *A. japonicus* output was up to 92,567 t (Ministry of Agriculture China, 2008). The outputs for the Shandong and Liaoning provinces were far ahead, with 60,689 and 29,034 t, respectively. The sea cucumber output of Fujian province was 1224 t, more than that of Hebei Province (1183 t), which made it the third *A. japonicus* producing area. In 2009, the national sea cucumber output was up to 102,159 t, and the major producing areas were still Shandong and Liaoning, with outputs of 62,792 and 36,134 t, respectively, whereas those of Shandong and Fujian were 1508 and 1338 t, respectively (Ministry of Agriculture China, 2009). In 2010, the national *A. japonicus* output was still growing with 130,303 t. Shandong, Liaoning, Fujian, and Hebei were the top four, with outputs of 66,300, 59,764, 2128, and 1643 t, respectively (Ministry of Agriculture China, 2010). In 2011, the main suppliers were still Shandong and Liaoning, with respective outputs of 71,011 and 54,954 t; Fujian and Hebei ranked third and fourth, with 7082 and 4024 t, respectively (Ministry of Agriculture China, 2011). Similarly,

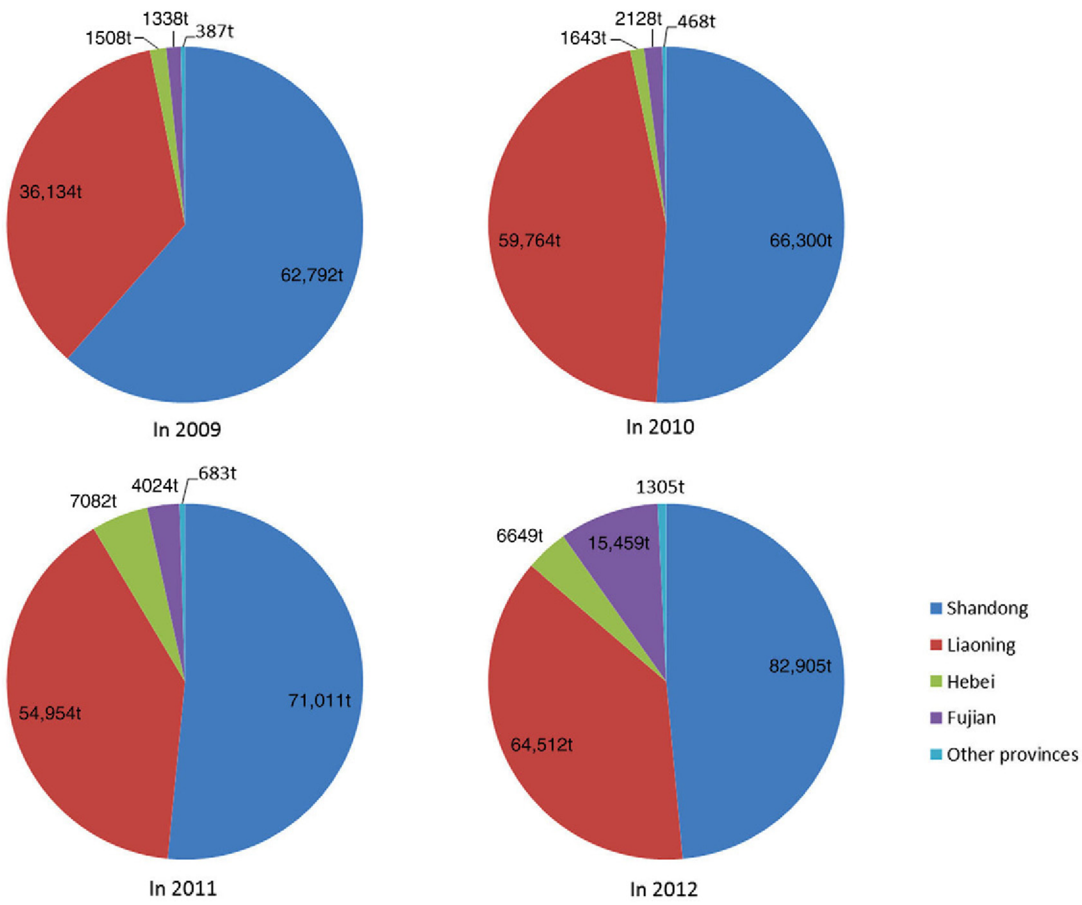

FIGURE 21.5

Production of *A. japonicus* (wet weight in tons) in major provinces of China in 2009–2012.

From Ministry of Agriculture China, 2009-2012

the main suppliers were Shandong and Liaoning in 2012, with respective outputs of 82,905 and 64,512 t (Ministry of Agriculture China, 2012) (Figure 21.5).

The Chinese market for the sea cucumber *A. japonicus* is divided into three sectors under the influence of the industry's development history and market competition. The northern sector with Dalian at its core is the first and leading market. It corresponds to the traditional sea cucumber aquaculture area revolving around *A. japonicus*; its development is tributary of a saturated market with a well-established and mature consumer market, a diversity of products available, and fierce market competition. The *A. japonicus* in Dalian always enjoys a high reputation. The saying goes "Good sea cucumber are produced in Dalian." Among the Dalian brands, Bangchuidao, Xiaoqin, and Zhangzidao are the most

famous ones. The second sector is another key market in the north, centered around Weihai, Yantai, and Qingdao. This one revolves around the sea cucumber *A. japonicus* produced in the Shandong province. With large-scale aquaculture industries, well-developed processing plants, and area expansion for culture, the market even developed some local brands, such as Oriental Ocean, Homey, Laoyijia, Gongpin, and Shuangju. In recent years, these local brands in Shandong have gained more market share than before with diversified products. Shandong, therefore, becomes another important part of the *A. japonicus* market, abreast of Dalian. However, because of the lack of promotion by the leading sea cucumber brands and their operation in the area, the Shandong market is scattered and disorganized. The third sector has become the fastest-growing in China over recent years and is centered around Fujian. Due to differences in climate, the growth period for *A. japonicus* has been shortened in Fujian and Zhejiang provinces, and thus the sea cucumber aquaculture has developed over a large scale. The southern sea cucumber market area is aimed at middle- and low-end mass markets by means of advantages on product research, process technology, and production cost. Because of its rapid development, the brand operation in this sector is still in the preliminary stage.

With the expansion of southern industries, Liaoning's sea cucumber production volume and value has fallen to some extent from 2010 to 2011. Production is experiencing a southward shift. The coastal culture area is constantly expanding; it has spread from Bohai and Huanghai to Donghai and Nanhai, thus promoting transformations in the traditional Chinese sea cucumber industry.

21.4.3 CONSUMER HABITS

With a history of several millennia, China has deep-rooted traditional concepts that influence its consumer and culinary cultures. Family values and ancestral culture are other important factors (see Chapters 1 and 2). Furthermore, economy and frugality are widespread concepts. All these embody the national character of the Chinese consumer culture.

Gift-giving etiquette is a unique feature of Chinese culture. Chinese people always send gifts on New Year's Day and during some festivals, as well as on weddings and funerals, to promote friendly relations. The type of gift that is suitable for an occasion is also carefully considered. An ideal gift can express some special wish and transmit information both to giver and receiver. Chinese people can encourage and educate each other, show knowledge and accomplishment, express friendship and compassion, and solidify their image in the eyes of others by sending suitable gifts. Gifts may also reveal a desire to be approved and understood by others, and can convey kindness and love. An appropriate gift can make the receiver happy, cater to his pleasure, and the gift also reflects the sender's status and taste. Therefore, high-end gifts can make the sender feel more honorable. In this context, the gift should not only be useful, but also be expensive. It is even better if there is some implied meaning in the gift, such as health tree, auspicious jade, wealth tree, and so on. The gifts with a positive moral message are very popular among Chinese.

Nowadays, health and environmental consciousness are advocated by many societies, including China. Hence, sea cucumbers serve the Chinese gift culture admirably, most especially *A. japonicus*, which is a renowned medicine and tonic, doubled with a delicious taste. Sending "spiky sea cucumbers" means sending health and best wishes to others. Moreover, they represent high-end gifts, which gives esteem to the sender. Gift etiquette in China drives a large proportion of sea cucumber consumption, especially highly prized products like *A. japonicus*. Luxurious packages have great value, and the prices range from one hundred to several thousand dollars (Figure 21.6).

FIGURE 21.6

Common *A. japonicus* products sold on the market in China: (a) retail dry sea cucumber; (b) dry sea cucumber packages.

Photos by Dongxue Xu

21.4.4 MARKET DEVELOPMENT

21.4.4.1 Product diversity

There are many types of products derived from *A. japonicus*, which can be divided into the following according to their origin: Chinese Jiaodong, Liaodong, and Donghai types; Japan; Republic of Korea; and Russia types, among others. They can also be divided into various processing methods, such as fresh, dried, half-dried, salted, pressure-cooked, and ready-to-eat. Finally, refined products include liquid sea cucumber, sea cucumber capsules, and sea cucumber peptides. Refer to Chapter 20 for details on processed products and cuisine.

Currently, dry sea cucumber is still the major product, but consumers have to convert this product back to the hydrated form before eating, after following a complicated procedure that is inconvenient for many consumers (see Chapter 20 for details on cooking methods). Hence, in recent years, instant or ready-to-eat sea cucumbers have become popular. Instant sea cucumbers packed in bags without any preservatives can be soaked in pure water and eaten directly and, moreover, nutrients like glycosaminoglycans for instance are fully preserved, and readily available (see Chapters 19 and 20). This kind of sea cucumber product not only maintains form, but also solves the difficult digestion and absorption problems related to some other *A. japonicus* products on the market.

21.4.4.2 Consumer groups

In the past, the main consumers of sea cucumbers were the upper-class middle-aged and elderly people. Long-term consumption can nourish the kidney and strengthen the essence, thus making middle-aged people more energetic at work. Eating sea cucumbers can also improve immunity and generally strengthen health.

The development of health consciousness and the growing economy means that more and more people have begun to eat sea cucumbers. Modern Chinese have changed their lifestyles and dietary habits considerably. Some diseases, such as diabetes, high blood pressure, and cancer, have exhibited

increasing rates in recent years. Eating sea cucumbers can play a role in preventing these chronic diseases. It is estimated that the consumer group will grow with the perception that sea cucumbers not only contribute to good health but may also enhance beauty and intelligence.

21.4.4.3 Market changes

The sea cucumber industry entered its golden age after the SARS epidemic in 2003. Sea cucumber markets expanded rapidly, both in terms of production and sales. With the booming sea cucumber industry, monopolies in sale systems developed in each city. However, 2012 was a bad year for the sea cucumber aquaculture industry in China. Some sea cucumbers developed skin diseases, largely due to poor culture methods; as a result, prices plummeted. In 2013, sugared dried sea cucumber came out and created a stir that influenced the whole sea cucumber market. Regulations issued by the Chinese government to promote frugality also exerted pressure on the sea cucumber market. All these circumstances created difficult times for the sea cucumber industry as a whole, especially the monopolies of the species (*A. japonicus* mainly) maintained in the sale markets. Many sellers were not able to make sufficient profits, and some closed down, while others turned to alternative commodities.

This downturn in the sea cucumber industry made a few sea cucumber enterprises question its development. In the early days, the market developed too fast, many sellers were busy expanding, and lacked supervision on exclusive shops in the end market, which led to a loophole in the sale channel. Because of this, some brand sellers understood the drawbacks of such unbridled development.

A tighter enforcement of regulations on sea cucumber markets will help consumers get to know their sea cucumber products more thoroughly, and the brand sellers will pay more attention to quality and brand image. Finally, the exclusive sale market will become a truly sustainable market. The core of the sea cucumber industry revolves around captive breeding and aquaculture, as wild stocks, especially those of *A. japonicus*, cannot sustain such huge commercial ventures. Therefore, with the development of the sea cucumber industry, the experience gained in captive breeding technology will open the end sale market gradually, and supply and demand will also achieve a new balance, all of which will help the industry mature and ultimately achieve significant but sustainable benefits.

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CHAPTER

Apostichopus japonicus: FISHERIES, TRADE, AND FOODWAYS IN JAPAN

22

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SUMMARY

Nearly all sea cucumbers *Apostichopus japonicus* currently harvested in Japan are wild, not cultured. This is one of the prominent characteristics of Japanese *A. japonicus* fisheries. Dried *A. japonicus* has been a major export commodity from Japan to China for at least 350 years. Japanese also consume sea cucumbers but they have developed a taste for them since the seventeenth century that differs from the Chinese model. This chapter explores the current sea cucumber fisheries in Japan: fisheries regulations, as well as landing and exporting data. Around the mid-2000s, new types of sea cucumber products, such as salt-preserved body wall, became common Japanese products to be exported to China. To confirm the new trend in the sea cucumber trade, an exploration of Japanese *A. japonicus* on the Hong Kong and Chinese markets is provided. Moreover, problems related to the shift of sea cucumber processing from dried to salt-preserved products is discussed. Finally, the chapter introduces efforts of local sea cucumber fishermen and processors to enhance the domestic sea cucumber market in order to maintain sustainable sea cucumber fisheries.

Keywords: *Apostichopus japonicus*; body walls; fisheries regulations; memorial service; salt-preserved; sea cucumber; Sino-Japanese trade; spiky variety

22.1 INTRODUCTION

In Japan, sea cucumbers or holothurians are generally known as *namako*. Strictly speaking, *namako* refers to fresh or live sea cucumbers, while the dried form (beche-de-mer) is called *iriko*. Etymologically, the word *nama* in Japanese means fresh, thus *nama-ko* refers to fresh or live *ko*. The meaning of the Japanese word *iri* is for the boiled or desiccated product and *iri-ko* thus refers to dried *ko*. Fermented intestine of sea cucumber prepared for human consumption is called *ko-no-wata*, meaning sea cucumber (*ko-no*) intestine (*wata*). Although there is no usage of the word *ko* in today's Japanese language, one can infer that *ko* was the ancient Japanese term for sea cucumber that can be declined as *namako* (fresh sea cucumber), *iriko* (dried forms), and *konowata* (intestine). Consequently, *konoko* (gonad) refers to *ko* (offspring) of *ko* (sea cucumber).

Since the eighth century, the people of Noto (present day Ishikawa Prefecture), Oki (Shimane Prefecture), and Shima (Mie Prefecture) delivered *iriko* as a tribute to the emperors (Kito, 2004; Amino, 2000). The book of laws and regulations, *Engishiki*, compiled in 927 A.D. mentioned *iriko* as a tribute

Akamine, J. 2015. Chapter 22. *Apostichipus japonicus*: fisheries, trade, and foodways in Japan. In: Yang, H., Hamel, J.-F., Mercier, A. (Eds.), The Sea Cucumber *Apostichopus japonicus*. History, Biology and Aquaculture, Academic Press, pp. 399–422. http://dx.doi.org/10.1016/B978-0-12-799953-1.00022-2

(Shibusawa, 1992). There being no way of preserving sea cucumbers except in the dried form *iriko* probably explains why it became a tribute for noble families and Shinto Shrines. Until the nineteenth century, wealthy Japanese often ate *iriko* but this tradition has been lost in contemporary Japan.

On the other hand, in the domestic market, sea cucumbers are commonly consumed raw in slices soaked in a mixture of vinegar and soy sauce. For contemporary Japanese, sea cucumbers are a seasonal delicacy only consumed in winter, especially during the winter solstice and the New Year, since it is believed that consuming sea cucumbers helps raise body temperature. Sea cucumbers can be consumed at home but they are often consumed in restaurants or Japanese-style bars (*izakaya*) in winter. Both *konowata* and *konoko* are rare and luxurious delicacies and they are only served in one or two restaurants as *kuchitori* (appetizers). They have a distinct flavor and an appealing color that go well with *sake*.

This chapter examines contemporary Japanese sea cucumber (*Apostichopus japonicus*) fisheries, trade, and foodways. Almost all of the *A. japonicus* currently harvested in Japan are wild rather than cultured. Sea cucumbers produced in aquaculture facilities in Japan are used chiefly to stock-enhance natural populations. This reliance on wild stocks is one of the prominent characteristics of *A. japonicus* trade in Japan. Moreover, since dried *A. japonicus* (*iriko*) has been a major export commodity from Japan to China for at least 350 years, some historical aspects of fisheries and foodways are covered here. Historically, dried sea cucumbers have been such an important commodity not only for the Japanese but also for other maritime Asian countries that it is important to analyze the current status of the Japanese sea cucumber industry in a wider Asian historical context to understand the global stakes of these fisheries. From the seventeenth century, the Japanese developed a taste for sea cucumbers that is quite different from that of the Chinese (see Chapter 1). At the time, the Tokugawa feudal government decided that dried sea cucumbers would be the main export item to China in return for silks and raw silk threads. The chapter discusses current sea cucumber fisheries: Japanese fisheries regulations, landing data, and exportation data. Not all maritime communities in Japan produce sea cucumbers. There are five distinct active sea cucumber fisheries areas in Japan, which have been intensively producing dried sea cucumbers since the seventeenth century or even before that. However, around the mid-2000s, new types of sea cucumber products, such as salt-preserved body wall, have been exported to China where they are further processed into a dried form. This new trend in the Japanese sea cucumber trade is described in detail, and the last part of the chapter points out the problems regarding the shift of sea cucumber processing from dried to salt-preserved products. A final segment introduces efforts of local sea cucumber fishermen and processors to enhance the domestic sea cucumber market in order to maintain sustainable sea cucumber fisheries. Portions of this chapter are based on published work by Akamine (2004, 2007, 2009, 2013).

22.2 HISTORY OF JAPANESE SEA CUCUMBER FISHERIES AND FOODWAYS

Apart from species found in subtropical waters in Okinawa (southern Japan), the most common species in the Japanese archipelago is *A. japonicus*. In his classic study, Choe (1963) also noted two other commercially harvested species in the temperate waters around the Japanese archipelago. These are *Parastichopus nigripunctatus*, called *oki-ko* (an offshore species), and *Cucumaria japonica*, called *kin-ko* (golden species) or *fuji-ko* (flower-like sea cucumber). Although Japanese landing statistics and international trade statistics do not distinguish species, it is the author's understanding that these two species currently are rarely harvested and thus only account for a small quantity of processed and traded sea cucumbers in Japan. In this chapter, sea cucumber thus refers only to *A. japonicus* except when otherwise mentioned.

The Chinese typically prefer the more spiky varieties of *A. japonicus*, which the traders in Hong Kong call *guandong-shen*, while the less spiky varieties are called *guanxi-shen*. The words *guandong-shen* and *guanxi-shen* literally mean "eastern variety of sea cucumbers" and "western variety of sea cucumbers," respectively in both Chinese and Japanese. In reality, *guandong-shen* refers to sea cucumbers harvested in the northeastern parts of the Japanese archipelago, namely Hokkaido and Aomori prefectures, while *guanxi-shen* refers to sea cucumbers harvested in the rest of the country. The former variety is two or three times as expensive as the latter variety in Hong Kong and Chinese markets. However, in the Japanese market, no particular price difference exists in regard to the spiky appearance of sea cucumbers. The body color of *A. japonicus* varies from red to green and black (see Chapter 3 for details about color variants). For raw consumption, as in *sashimi*, the red one (*aka-namako*) is preferred and its price is almost double compared to that of the green (*ao-namako*) and black (*kuro-namako*) variants in Japan. The red variety lives in reef areas precluding the use of dredge nets for their collection. Thus, the *ama* divers pick them up manually. The female *ama* divers in Shima (present day Mie Prefecture) and Noto (Ishikawa Prefecture) are particularly famous nationwide. Once dried, the red variant takes the form of a rugby ball and receives a low market value (because the market prefers slender products). Inversely, the black variant has no market value for *sashimi*-oriented domestic markets because of its unappetizing color, while it receives a high market value in China when dried. Most of the processors, therefore, prefer to process the green and black variants into dry product for export.

22.2.1 SEA CUCUMBER FOODWAYS IN JAPAN

There is no document available describing how the ancient noble Japanese cooked the offered *iriko* in the eighth and ninth centuries. As a side note on cooking methods, Okumura Ayao, a well-known contemporary scholar in the field of Japanese foodways, inferred that *iriko* was rehydrated in water and cooked with seasonings, such as salt, soy sauce, and vinegar (Tsuboi, 1985). It is only in the seventeenth century that sea cucumber dishes became well documented. A pioneering Japanese cookbook, only available among the nobility, titled *Ryori Monogatari* (Cuisine Story) published in 1643, outlines recipes for the four types of sea cucumber products: *namako*, *konowata*, *konoko*, and *iriko* (Hirano, 1988). Table 22.1 summarizes all 13 sea cucumber recipes illustrated in *Ryori Monogatari*. The recipes for fresh sea cucumber (*namako*) dishes are *namasu*, *fukura-iri*, *kodatami*, *suko*, and *irisake-kake* (Hirano, 1988). *Namasu* is a general term for a dish of raw fish and vegetables seasoned with vinegar. To prepare the dish *fukura-iri* (boiled dish), they used chopped sea cucumbers briefly immersed in boiling *dashitamari* (Hirano, 1988). To prepare *kodatami* (*namako* broth), sliced sea cucumbers were immersed in *sake* and added to a *dashi* soup flavored with salt and *mirin*, accompanied by *wasabi* (Hirano, 1988). *Suko* refers to sea cucumber pickled in vinegar (Hirano, 1988). *Namako* shredded into slivers are poured in *irisake* to produce *irisake-kake*. Five recipes for *iriko* are *atsumejiru* (*iriko* broth), *kezurimono* (sliced *iriko*), *zororiko* (boiled *iriko*), *ao-ae* (*iriko* with mashed beans), and *mizu-ae* (*iriko* with vegetables or other ingredients) (Hirano, 1988). All sea cucumber recipes constituted high-end dishes and were included in menus for such events as tea ceremonies. These cooking techniques were applied in the pursuit of delicious tastes with a gourmet approach.

Japanese medicine was greatly influenced by Chinese medicine, which developed during the Ming Dynasty (1368–1644), following a scholarly approach based on the scientific structures of various food ingredients. However, Japanese medicine not only followed Chinese medicinal science, but also developed its own, starting in the seventeenth century. This independent attitude is clearly seen in

Table 22.1 Sea Cucumber Dishes as Found in *Ryori Monogatari*[a]

Type of Dish	Type of Sea Cucumber	Number of Recipes	Name of Dish
Broth	*namako*	1	*kodatami*
	iriko	1	*atsumejiru*
Clear soup seasoned with vinegar	*konowata*	1	*suimono*
	namako	2	*namasu, suko*
	iriko	2	*aoae, mizuae*
Salad with *irisake*[b]	*namako*	1	*irisakekake*[b]
	konoko	1	*irisakekake*[b]
Boiled	*namako*	1	*fukurairi*
	iriko	1	*zororiko*
Snack	*iriko*	1	*kezurimono*
Salt-fermented	*konowata*	1	*konowata*

[a]Ryori Monogatari *provides no particular name but a name is given by the author.*
[b]irisake*: a liquid seasoning prepared by boiling down a mixture of sake and soy sauce, fish stock, pickled plums, and other ingredients.*
Adapted from Hirano, 1988

descriptions of sea cucumbers: Chinese literature typically focuses on dried products and hardly pays attention to fresh forms of sea cucumber, such as intestines and reproductive organs (gonads), which are well discussed in Japanese documents. *Waka Shokumotsu Honzo* (Japanese Medicinal Foods in Tanka[1]) published in 1643 is probably the first medicinal book to distinguish the medicinal functions of *namako* (fresh sea cucumber) and *iriko* (dried sea cucumber) saying that they make the body cold and warm, respectively (Amano and Hanawa, 2012). *Eppo Shokumotsu Honzo* (Eppo's Medicinal Foods) published in 1671 mentions *konowata* (fermented sea cucumber intestine) for the first time in the history of medicinal books, describing its properties against phlegm. The 12-volume *Honcho Shokkan* (Our Country's Food Encyclopedia) published in 1695 is a monumental work about Japanese medicine. This is the first medicinal book dealing with all four types of sea cucumber: *namako*, *iriko*, *konowata*, and *konoko*. These medicinal books published in the seventeenth century are not a translation of Chinese medicine, but a compendium of numerous scientific reflections from a Japanese standpoint.

As can be seen, there was a variety of methods for cooking sea cucumber in the Edo period (1603–1868) but Japanese recipes for dried sea cucumbers (*iriko*) differed substantially from Chinese versions, where *iriko* were often cooked with meat. In addition, in present-day Japan, *iriko* is rarely consumed, contrary to China where it represents the main form of sea cucumber consumed (see Chapter 20 for Chinese sea cucumber cuisine). In fact, most Japanese do not know *iriko*. Why Japanese *iriko* dishes declined in popularity in present-day Japanese cuisine remains unclear. One possible reason is that the Japanese government during the Meiji period (1868–1912) tried to expand its wealth, military power, and modernity by exporting *iriko* to China rather than keeping it for domestic consumption. Another possible reason is

[1]A *tanka* is a 31-syllable Japanese poem.

a change of taste among Japanese consumers after the Second World War. Ishikawa Naoko, a Japanese scholar in foodways, states the school lunch system introduced by the USA after WW II, based on bread and milk, promoted the "westernization of food" in Japan (Ehara et al., 2009). The westernization also affected the lifestyle, especially during the postwar recovery. This State-guided program included a shift from the prewar carbohydrate-centered meals to meals that supplied more protein and fat. Japanese currently prefer greasy meals so that the simple taste of *iriko* foods may not appeal to them any longer.

22.3 EXPORT OF JAPANESE DRIED SEA CUCUMBER (*iriko*) TO CHINA BETWEEN THE SEVENTEENTH AND NINETEENTH CENTURIES

Japan had been producing and consuming dried sea cucumbers when China's market expanded in the late sixteenth century or the early seventeenth century. However, this does not mean that Japan simply exported the surplus out of its domestic market. At that time, the Tokugawa feudal government not only encouraged coastal people in the existing maritime communities to produce more *iriko* for export but also encouraged newly opened ones in Hokkaido, in the northernmost part of the Japanese archipelago (then called *Ezo*), to become major *iriko* harvesting zones, mainly for export purposes. The reason Hokkaido was chosen as a major production site was twofold. First, the cultural minority, the Ainu, who lived in Hokkaido, provided a source of labor for *iriko* production (the Ainu were often forced into such work). Second, *iriko* produced in Hokkaido had more spikes than that found elsewhere in Japan, a factor highly appreciated by the Chinese (*A. japonicus* variants with sharp spikes from Hokkaido and Aomori prefectures is currently called *guandong-shen* and highly prized by Chinese traders) (see Chapter 4 for *A. japonicus* anatomy).

Under Tokugawa's trade monopoly regulations, *iriko* trade was centralized in Nagasaki, an official trading port, and classified into 10 categories depending on its quality and size, as shown in Table 22.2.

Table 22.2 Classification of Dried Sea Cucumber During Edo Period

Classification	Number of Pieces per 600 g	Weight per Piece (g)	Standard Length (mm)
10	10	60	136
9	12–13	46–50	121
8	20	30	106
7	30	20	91
6	40	15	76
5	55	11	61
4	80	7.5	45
3	120–130	4.6–5	>30
2	150–160	3.8–4	30
1	200–300	2–3	<30

From Nippon Suisan Seihinnsi (Japanese Marine Products)

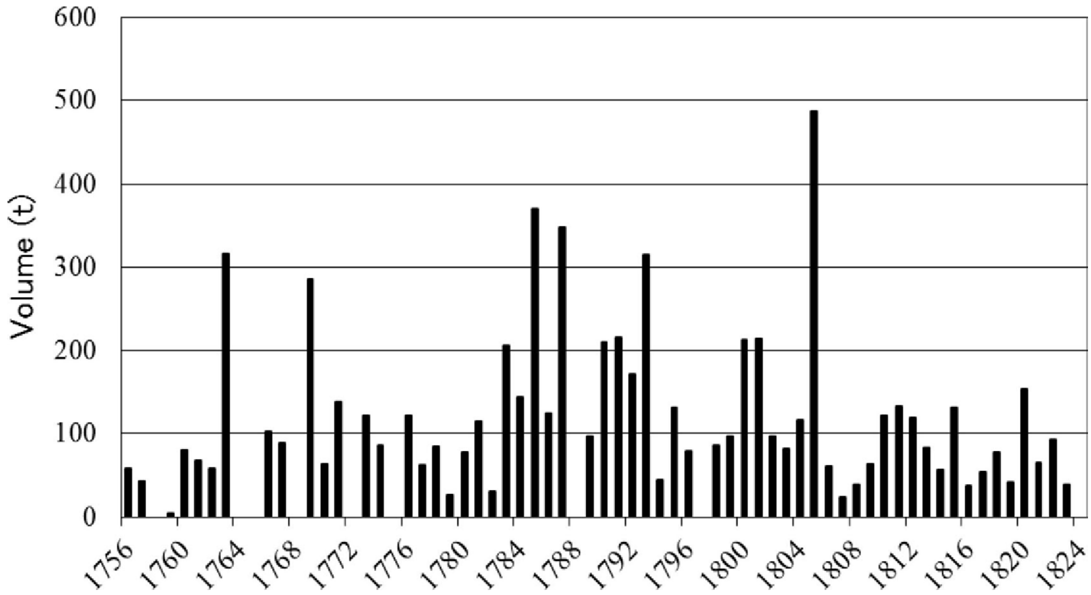

FIGURE 22.1

Dried sea cucumber exports from Japan to China from 1756 to 1823.

From Nagazumi, 1987

Iriko was not only the top export commodity in value, followed by dried *kombu* or kelp (*Saccharina* spp.), but was also the second most exported commodity in volume after *kombu*. Figure 22.1 illustrates the trend of *iriko* exports from Japan in the seventeenth and eighteenth centuries based on *Catalogue of Imports and Exports in Chinese Trades* (Nagazumi, 1987). A Japanese historian, Nagazumi Yoko, investigated Dutch archives on Japanese trade with China as unfortunately only few records on Sino-Japanese trade remains in both Japan and China. Interestingly and fortunately, the Dutch were so concerned about Chinese competitors that they intensively recorded information about the Sino-Japanese trade.

There is no doubt that a considerable amount of *iriko* was exported every year, but data for some years were missing. Only the number of straw bags, known as "hyo" rather than the actual volume *kin* (600 g) or *pikul* (60 kg), was marked on the original record and this makes it impossible to include accurate data in Figure 22.1. For example, in 1788, a total of 4630.5 bags of *iriko*, at least, were exported to China by 11 ships. The bag was originally used to pack rice but its weight for *iriko* would be equal to 120 *kin* (72 kg). Thus, the number is estimated at 333.4 metric tons of *iriko* exported in that year.

Although the Dutch records are not complete, they can greatly help to reconstruct a picture of the Sino-Japanese sea cucumber trade. For example, in 1763, the volume of exported sea cucumber reached 317 metric tons. This was the year when imports of gold and silver from China to Japan began (prior to this, Japan exported gold and silver instead). Since the Tokugawa government did not have sufficient copper in exchange for the precious metals, more *iriko* was needed to complete the trades

with China. In 1785, the Tokugawa government began to appoint fishermen throughout the archipelago to collect more *iriko* and exports reached 371 metric tons (Arai, 1975). Thereafter, production continued to increase and exports reached a record high of 487 metric tons in 1805.

Tokugawa's monopoly in the *iriko* trade lasted until 1865, after which European and Chinese traders joined in this lucrative trade. Two years later, Japan experienced the Meiji Restoration and free trade began. Under the Meiji Government, *iriko* continued to be one of the major profitable commodities exported. This situation encouraged the Japanese Government to open more fishery grounds in the Hokkaido area. Migrants, mostly from Honshu islands, came to Hokkaido to take part in the fishery industry.

22.4 SEA CUCUMBER FISHERIES IN CONTEMPORARY JAPAN

22.4.1 FISHERIES LAW SYSTEM IN JAPAN

To understand Japanese sea cucumber fisheries, it is necessary to explore the fisheries system regulated under the Fishery Act since 1949. The basic principle of the law is to maintain order in fisheries through the "fishery rights" that are strictly applied in coastal communities involving a "fishery permit" system. The law dictates that important decision-making be administrated by fishery adjustment agencies, such as the Fishery Adjustment Commissions (FAC), which are mainly composed of fishery operators and employees.

22.4.1.1 Fishery rights

In order to maintain order and adjust fishery operations among operators, a system of fishery rights over the public waters has been established. The fishery rights, controlled by prefectural governors, allow certain fisheries to be operated exclusively in specific waters. Only local fishery cooperative associations (FCA) are eligible for concessions. To regulate the application and implementation of the fishery rights, local FCAs have predetermined regulations, i.e., target species, fishing seasons, and harvesting methods. The FCAs also oversee the fishing activities conducted by individual members.

The fishery rights can be divided into three categories: common fishery, fixed gear (set-net) fishery, and demarcated fishery (aquaculture). The first category is further classified into three types: (1) a fishery operated to harvest seaweed; (2) a fishery operated to harvest shellfish, or (3) a fishery operated to harvest other stationary aquatic animals designated by the Minister of Agriculture, Forestry, and Fisheries. The sea cucumber is one of the animals classified in the third category. Thus, no one can collect sea cucumbers without fishery rights.

22.4.1.2 Fishery permits

Certain types of fisheries require a fishery permit. However, a fishery permit is different from the application of the fishery rights. Issuing a permit means an administrative action to lift interdiction in a specific case when required in order to manage–conserve marine stocks. Not all types of fisheries require a fishery permit but almost all economically important species do. There are two types of permits: those issued by the Minister of Agriculture, Forestry, and Fisheries and those issued by the prefectural governor. The latter type, called a "Governor Permitted Fishery (GPF)," observes the regulations of each prefecture. Small-scale trawl fisheries that employ a powered vessel of less than 15 gross tons are regulated under the GPF. Dredge net fishery for sea cucumbers also falls under this category. The

GPF is valid for 10 years. To renew the GPF, the fishing plan must be discussed with the prefectural government. For conservation reasons, it is more difficult to apply for new permits than for renewal of existing ones.

22.4.1.3 Fishery adjustment system

There are two types of fishery adjustment commissions: the Sea-area Fishery Adjustment Commission (SFAC) and the Broad-area Fishery Adjustment Commission (BFAC). Taking fishery conditions into consideration, the Minister of Agriculture, Forestry, and Fisheries divides the ocean surrounding Japan into 66 sea-areas. In principle, an SFAC is set up for each sea-area in each prefecture and is under the authority of the prefectural governor. Each sea-area is a unit subjected to fishery management. The SFAC is generally composed of 15 commissioners, of which nine are elected among fishermen, and six (four academics with fishery expertise and two people representing public interests) are appointed by the prefectural governor. The SFAC plays an advisory role in all matters handled by the administrative agency in regard to fishery rights and fishery permits. As for the GPF, each prefectural fishery regulation stipulates that a governor has to consult with the SFAC before granting a permit. In addition, the SFAC has the authority to make decisions concerning the arbitration, instruction, and authorization of the permit.

According to the Fishery Act in Japan, regardless of commercial or self-consumption purposes, no one without fishery rights can collect sea cucumber, because the animal is designated as a species regulated by common fishery rights. Even with the fishery rights, those who wish to harvest sea cucumbers with dredge nets have to apply to the prefectural governor for a fishery permit because dredge net fishery is designated as a GPF (although the fishery permit is not necessary to dive-harvest sea cucumbers). Thus, the prefectural governor, together with the SFAC, plays an important role in managing sea cucumber harvests in Japan.

22.4.2 SEA CUCUMBER FISHERIES IN CONTEMPORARY JAPAN

A. japonicus can be found nearly everywhere around Japan, except in the tropical waters of Okinawa. Figure 22.2 illustrates five major harvesting areas of *A. japonicus* based on catch data from 2006, when a total of 19 prefectures produced >100 metric tons of fresh sea cucumber. Among them, 17 prefectures are on the list (Table 22.3). All of them have been renowned sea cucumber producing sites since the Edo period. Traditional *iriko* production sites from the eighth century, such as Shima, Oki, and Noto, are also on the list. It is noteworthy that Hokkaido's 2725 metric tons of sea cucumber catch in 2006 accounts for 26% of the national production, Aomori for 17%, and Yamaguchi for 10%, accounting for 53% of whole national production. The top five prefectures in 2006 produced 6502 metric tons of sea cucumbers, accounting for 63% of the total national production. The 10 main prefectures in 2006 produced 8260 metric tons of sea cucumbers and accounted for 80% of total production.

Unfortunately, there are no official statistics available illustrating the current sea cucumber fisheries in Japan. The Japanese Government enacted administrative and financial reforms in the 2000s and gave up collecting national sea cucumber landing statistics; hence, 10,344 metric tons in 2006 was the last record. Figure 22.3 shows Japanese sea cucumber landings from 1894 to 2006. Sea cucumber catches reached a peak in 1968, when 13,023 metric tons were collected, and were at their lowest in 1993, at 5996 metric tons. In the 1960s, while the Japanese economy enjoyed rapid growth based on industrial production, Japanese fisheries declined due to lack of manpower in fishing villages. Decline of sea

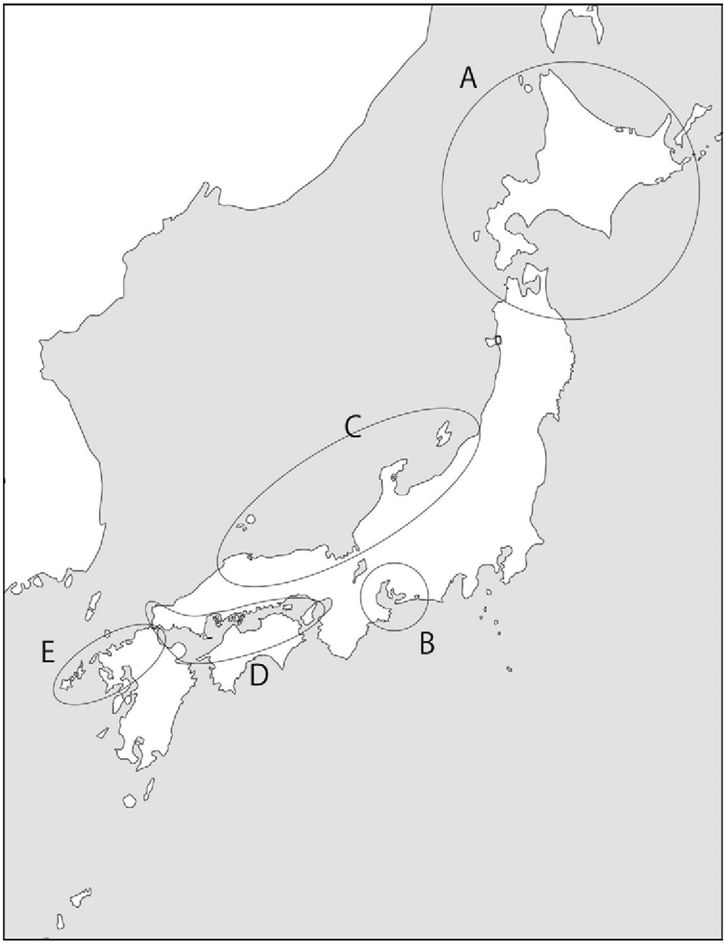

FIGURE 22.2

Five major sea cucumber producing areas in Japan. Northern Japan (A), Ise-Shima (B), Noto (C), Setouchi (D), and northwestern Kyushu (E).

Adapted from Tsurumi, 1990

cucumber harvests after 1968 may be partly attributable to population outflow from fishing villages to industrial cities.

The reason why sea cucumber production in Japan has been increasing since 1993 is not clear. It can possibly be explained by the increasing Chinese demands for sea cucumber products. However, in the early 1990s, the Japanese economy enjoyed a so-called "bubble economy" when rich epicureans looked for novelty. Both *konowata* (fermented sea cucumber intestines) and *konoko* (dried sea cucumber gonad) are two such expensive delicacies that epicureans appreciated. Sales from *konowata* and *konoko* paid all the expenses of sea cucumber processing, including labor and raw material cost. Thus, sales of sea cucumbers either fresh or dried made net profits.

Table 22.3 Five Major Sea Cucumber Producing Areas in Japan

Type of Sea Cucumber	Sea Cucumber Cultural Zone	Major Prefecture	Catch (t)	Order of Catch
Guandong-shen				
A	Northern Japan		***4458***	
		Hokkaido	2725	1
		Aomori	1733	2
Guanxi-shen				
B	Ise-Shima		***555***	
		Mie	340	9
		Aichi	215	11
C	Noto		***992***	
		Niigata	400	6
		Ishikawa	363	8
		Shimane	104	18
		Kyoto	125	16
D	Setouchi		***2661***	
		Yamaguchi	1005	3
		Hyogo	525	4
		Ehime	395	7
		Oita	260	10
		Hiroshima	203	12
		Tokushima	152	15
		Okayama	121	17
E	Northwestern Kyushu		***362***	
		Nagasaki	514	5
		Fukuoka	102	19

From Fisheries and Aquaculture Production Statistics in Japan, 2006

Figure 22.4 illustrates the average price for fresh sea cucumber at the Senhoshi Fisheries Cooperative Association (SFCA) on Rishiri Island, northern Hokkaido, and may also provide some explanation for the increasing demand for sea cucumber products since 2003. It is in spring and summer that sea cucumber fisheries are practiced in Hokkaido, while they occur only during winter in other parts of Japan. Since sea cucumber, especially *namako*, is a winter specialty, there is little market demand for fresh sea cucumber throughout the rest of the year. According to processors in Hokkaido, sea cucumber intestines are easily damaged when manipulated, so *konowata* is a difficult product to process (Figure 22.5). Almost all sea cucumbers harvested in Hokkaido are for exportation. The prices before 2000 in SFCA

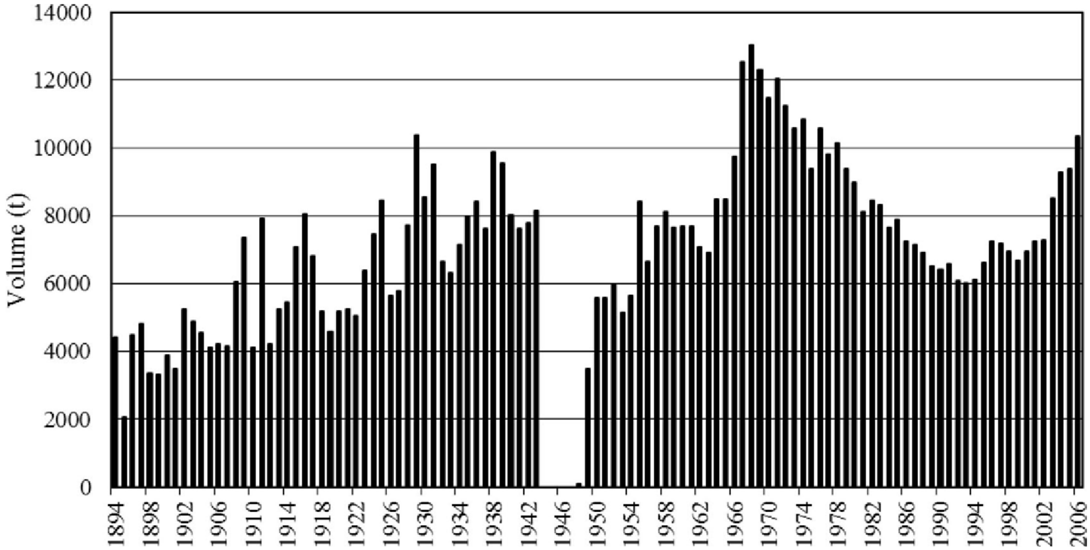

FIGURE 22.3

Sea cucumber catch in Japan (1894–2006).

From Ministry of Agriculture and Trade Statistics and Fishery and Mariculture Production Statistics

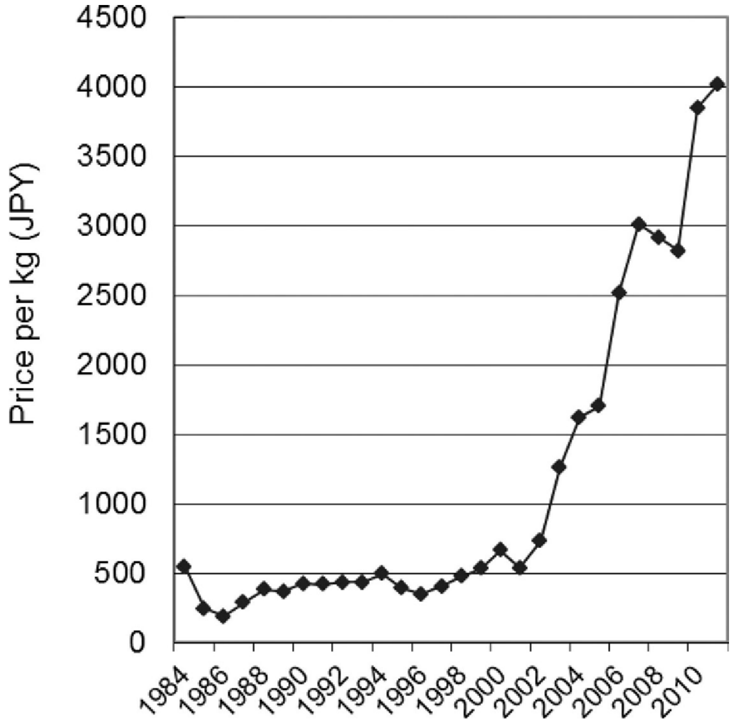

FIGURE 22.4

Average price per kilogram (JPY) of fresh sea cucumbers at the SFCA (1984–2011).

From SFCA Business Report (1980–2007) and Hokkaido Current State (2008–2011)

FIGURE 22.5

Processing *konowata* (intestine) and *konoko* (gonad) in a sea cucumber facility in Noto, Japan.

Photo by J. Akamine

were relatively stable while sea cucumber processors in mainland Japan enjoyed the *konowata* and *konoko* boom in the early 1990s (Figures 22.6–22.8). Sea cucumbers harvested in SFCA began to increase after 2001. Between 2002 and 2003, the average price of fresh sea cucumber at SFCA increased 1.7 times. The price of fresh sea cucumber at SFCA increased by a factor of 7.4 between 2001 and 2011.

Trade statistics showing Hong Kong's import of Japanese sea cucumber are illustrated in Figure 22.9. Hong Kong's imports of Japanese sea cucumber have increased steadily since 1997 (see also Chapter 21). Apart from 2001 and 2004, the mean value per kilogram continuously increased since 1996. In the late 1990s, Japan faced a depression after the bubble economy collapsed and only a few gastronomes continued to pay attention to *konowata* and *konoko*. Health-conscious foodies began to spread and consumers started avoiding salt-fermented *konowata* in order to decrease salt in their daily diets. Consequently, the Chinese demands for the Japanese sea cucumber could be the main driver that stimulated the sea cucumber fisheries in the late 1990s and onward in Japan.

In 2007, the import volume of Japanese processed sea cucumbers into Hong Kong reached 584.5 metric tons, which is 1.8 times more than the previous year (319.6 metric tons). On the other hand, the mean value of Japanese processed sea cucumber imported into Hong Kong in 2007 was 2075 HKD

FIGURE 22.6

Fresh *konoko* (gonad) in Noto, Japan.

Photo by J. Akamine

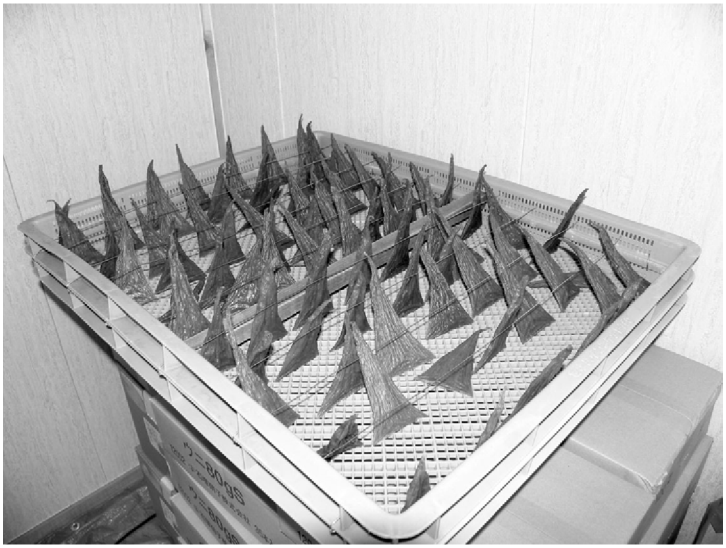

FIGURE 22.7

Processed dried *konoko* (gonad) in Sado, Japan.

Photo by J. Akamine

FIGURE 22.8

Several sea cucumber products available in a retail store in Noto, Japan, such as dried gonads, rehydrated sea cucumber cooked with soy sauce, and intestines with salmon roe in soy sauce.

Photo by J. Akamine

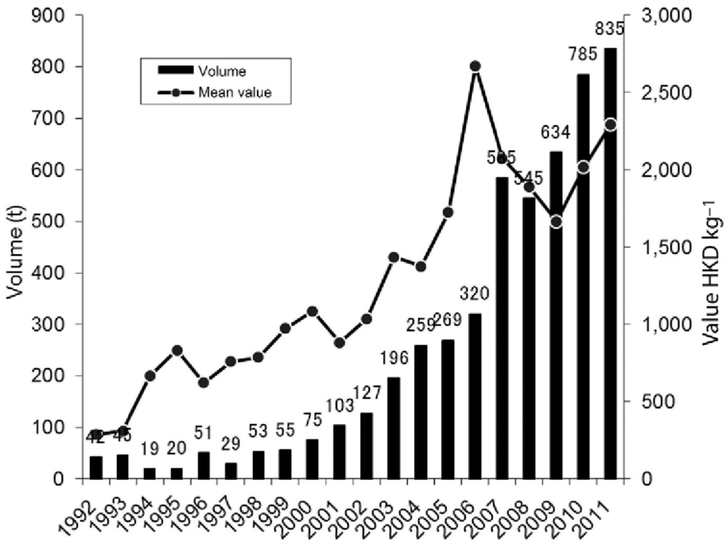

FIGURE 22.9

Sea cucumber imports into Hong Kong from Japan (1992–2011).

From Hong Kong Monthly Digest of Statistics

(260 USD) per kilogram, a decrease by more than 20% from 2006's value (2671 HKD or 340 USD). The decline of the mean value of imported Japanese sea cucumbers into Hong Kong was very clear in 2009, down to 1667 HKD (210 USD) per kilogram, representing a decrease of 40% compared to 2006.

Might the cause of this decrease be related to an oversupply of sea cucumbers on the market? Placing the yield rate at 5%, 2000 metric tons of fresh sea cucumbers would produce 100 metric tons of dried products. With a yield rate between 3 and 5%, the increase of 314 metric tons of dried sea cucumber in 2009 compared to 2006 required at least an extra 6,000 to 10,000 metric tons of catch (fresh weight). However, Japan had already caught 10,000 tons of sea cucumbers in 2006 and it would not be plausible to expect an extra 6,000 metric tons or more in such a brief period, considering that the largest catch in Japanese history was 13,000 tons in the 1960s when three times more fishermen were engaged in this fishery. According to a census taken from the Japanese Ministry of Agriculture, Forestry, and Fisheries, there were 593,830 fishermen in 1968, while today there are only 212,470 fishermen in Japan.

In fact, an item numbered 0307-9930 in Hong Kong's trade statistics is "beche-de-mer, dried, salted, or in brine." Strictly speaking, the item contains not only dried sea cucumber but also sea cucumber that was salt-preserved or preserved in brine, which are heavier due to a higher water content. Although the actual condition of the dried sea cucumber trade between Hong Kong and Japan is not clear, it is inferred that compared to the previous year, more salt-preserved or brine-preserved products were exported from Japan to Hong Kong since 2007.

Figure 22.10 shows dried sea cucumber exports from Japan to Hong Kong from 2004 to 2011, based on information from the Japanese Ministry of Finance (Table 22.4). It is only in 2004 that dried sea cucumber appeared in the Japanese trade statistics. In 2002, the CITES (Convention on International

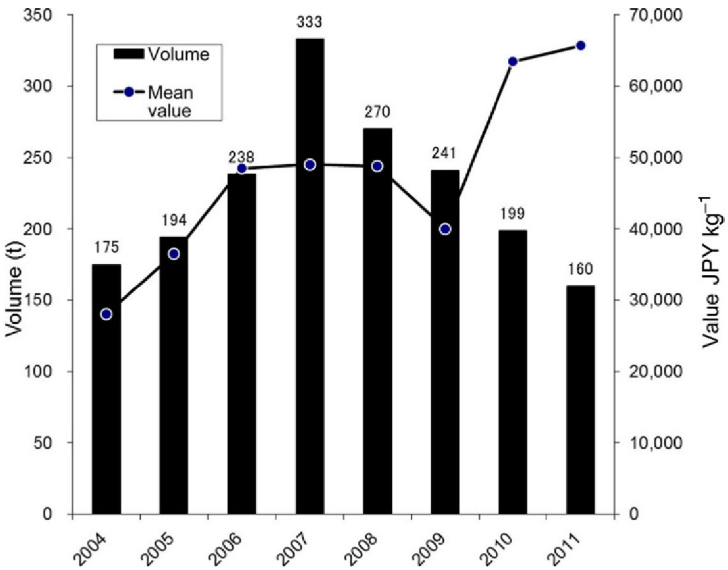

FIGURE 22.10

Dried sea cucumber exports from Japan to Hong Kong (2004–2011).

From Trade Statistics of Japan

Table 22.4 Japanese Exports of Dried Sea Cucumber (2004–2012)

Country		2004	2005	2006	2007	2008	2009	2010	2011	2012
Canada	*Q* (t)	0	0	0	0	0.0	0.5	0	2.8	0
	V (JPY kg^{-1})	n/a	n/a	n/a	n/a	40,913	9,573	n/a	8,337	n/a
China	*Q* (t)	13.9	18.6	27.7	1.6	0.5	0.4	2.8	30.0	38.4
	V (JPY kg^{-1})	23,365	27,944	33,250	28,170	34,035	26,538	29,447	39,300	34,083
Hong Kong	*Q* (t)	174.9	193.7	237.6	333.0	270.0	241.1	199.3	159.6	178.1
	V (JPY kg^{-1})	28,053	36,513	48,488	49,067	48,820	40,067	63,433	65,689	52,025
Macao	*Q* (t)	0	0	0	0	0	0	0	0.1	0.1
	V (JPY kg^{-1})	n/a	n/a	n/a	n/a	n/a	n/a	n/a	79,190	73,786
Malaysia	*Q* (t)	0	0	0	0.0	0.7	0	0	0	0
	V (JPY kg^{-1})	n/a	n/a	n/a	43,933	7,084	n/a	n/a	n/a	n/a
Singapore	*Q* (t)	0.1	0.0	1.6	1.2	1.2	0.4	0.4	0.4	0.6
	V (JPY kg^{-1})	20,360	0	22,330	61,135	31,862	38,372	35,904	83,070	31,407
Republic of Korea	*Q* (t)	2.8	7.5	4.9	6.5	8.4	2.7	0.8	0.7	0.4
	V (JPY kg^{-1})	19,562	25,053	7,842	20,973	7,777	8,611	14,984	20,238	14,454
Taiwan	*Q* (t)	30.6	1.5	0.8	2.0	2.0	3.6	5.5	1.5	0.9
	V (JPY kg^{-1})	6,656	23,010	53,501	27,750	3,654	3,839	4,175	11,791	4,475
USA	*Q* (t)	0.5	8.7	0.2	0.6	0.1	0.2	0.2	0.2	0.4
	V (JPY kg^{-1})	11,876	8,253	26,225	31,368	25,813	40,880	65,766	87,498	52,994
Total	*Q* (t)	222.7	230.0	272.8	344.9	282.9	248.8	209.0	195.4	218.8
	V (JPY kg^{-1})	24,680	34,286	46,057	48,329	47,072	39,123	61,191	60,310	48,567

Data shown as quantity (Q) in metric ton (t) and value (V) in Japanese Yen per kilogram (JPY kg^{-1}).
From Trade Statistics of Japan

Trades in Endangered Species of Wild Fauna and Flora) started to investigate sea cucumber status worldwide. In response to the request from CITES, the Japanese trade statistics reserves developed a category numbered 1605-90-930 only for dried sea cucumbers. Comparing the Japanese trade statistics to those of Hong Kong, the volumes appearing in the latter were always larger. This is probably due to the fact that Hong Kong statistics contained dried, salt-preserved, and brine-preserved products under the same category (0307-9930, "beche-de-mer, dried, salted, or in brine"). A closer look at both statistics revealed interesting facts. The average differences between Japanese and Hong Kong statistics during 2004 and 2006 were about 80 metric tons (84, 75, and 82 tons, respectively). Most of the differences are attributable to the heavier weight of salt-preserved and brine-preserved sea cucumber imported into Hong Kong. On the other hand, the differences between the two statistics increased after 2007; 252 tons in 2007 and 675 tons in 2011. The data clearly indicate a possibility that Hong Kong began to import more salt-preserved sea cucumber from Japan since 2007.

According to the Japanese trade statistics, the mean value of dried sea cucumber exported from Japan increased for several years except in 2009. This sudden decline could be attributable to China's economic slump in 2009 influenced by the Lehman Shock. This is probably because the Chinese market constantly demands certain levels of Japanese dried sea cucumbers instead of salt-preserved ones. During fieldworks in 2009 and 2010, the author of this chapter heard that almost all processors in Hokkaido and Aomori prefectures turned to producing the semifinal product, salt-preserved sea cucumber, and only a few of them still produced the final dried products.

The Japanese statistics only date back eight years of trade and therefore it is difficult to detect any trends (Table 22.4). Despite these limitations, four main characteristics of the Japanese export for dried sea cucumber can be highlighted: (1) Japan exports dried sea cucumbers only to nine countries and territories; (2) among them, Hong Kong is the major market of Japanese dried sea cucumbers; (3) the mean value of exported Japanese sea cucumber fluctuates (Taiwan bought the most expensive products in 2006, Singapore in 2007, and the United States after 2009); and (4) China purchased sea cucumber at a stable price (the average price for eight years was 30,256 JPY kg^{-1}, about 300 USD kg^{-1}).

The most expensive product made from *A. japonicus* in Japan comes from Hokkaido and Aomori prefectures. It is called *guandong-shen* and its production is limited to the northern part of the Japanese archipelago (Hokkaido produced 2725 metric ton of fresh sea cucumbers and Aomori Prefecture 1733 in 2006). Many buyers from Japan and China desire such premium varieties of *A. japonicus*. Since its production is limited, it fetches a high price. For example, the price of dried sea cucumber from Hokkaido fetched at 110,000 JPY kg^{-1} (about 1100 USD kg^{-1}) in November 2012. Traders compete to purchase small quantities of even a few kilograms of dried *guandong-shen*. Unlike Hong Kong where all varieties of dried sea cucumber are found (cheap or expensive products), Taiwan, Singapore, and the United States imported only small volumes of products in high demand. It is conceivable that some of them were reshipped to Hong Kong or China at a premium price. These complicated situations of dried sea cucumber trade from Japan emerged after 2006, when many processors shifted from production of dried sea cucumber to exportation of salt-preserved sea cucumber products.

22.5 JAPANESE *A. japonicus* IN HONG KONG AND CHINESE MARKETS

22.5.1 BUSINESS NETWORK BETWEEN NAM PAK HONG IN HONG KONG AND YIDE LU IN GUANGZHOU

Hong Kong is the international hub for sea cucumber trade. In 2011, it imported 5798 tons of sea cucumbers from 64 countries and regions and reexported 5722 tons to 14 countries. The amount reexported specifically to China was 5471 tons, or over 95.6% of the total volume exported from Hong Kong. According to dried marine product traders in Hong Kong, even more products are exported to China through various unofficial channels. Many traders agreed that only a small percentage, perhaps a few tons, of dried sea cucumbers were actually consumed in Hong Kong.

In Sheung Wan District (Hong Kong Island), a thriving business area called Nam Pak Hong exists, lined with dried seafood wholesalers and dealers of Chinese medicinal dried products. Nam Pak Hong is an area where a series of shops handle miscellaneous goods, but no map of Hong Kong indicates its location. The central areas of Nam Pak Hong are Bonham Strand West St. and Wing Lok St. In a broad sense, Nam Pak Hong includes Des Voeux Road West, where dried seafood retailers are concentrated. The Chinese counterparts of Nam Pak Hong traders in Hong Kong are concentrated in Yide Lu

(Guangzhou City, Guangdong Province). China imposes high tax rates on imported luxury products; for dried sea cucumbers, it is as high as 30%. According to stakeholders in the sea cucumber business in Hong Kong, traders use various methods to avoid this Chinese tax. Helpful in this regard are several sea routes and personal and commercial networks that were established before the Second World War and have brought traders in Nam Pak Hong and Yide Lu together. Given this historical and cultural background, it can be inferred that the actual amount of sea cucumbers exported to China from Hong Kong is larger than what is statistically reported. Sea cucumbers are distributed throughout China by means of Guangzhou-centered airline networks and road networks covering all of China.

The merchants in Nam Pak Hong have been reluctant to disclose information about their clients because of protocols governing business-related confidentiality. However, traders in Hong Kong who had in the past worked in a fragmented manner, started finding it preferable to team up, as sharks and sea cucumbers became subject to CITES. For example, wholesalers in Hong Kong have been warning Japanese fishermen, processors, and distributors to proceed with caution despite their euphoria stemming from the recent sea cucumber bubble in Japan (see Figures 22.9 and 22.10 for Japanese export of sea cucumbers to Hong Kong). Drawing on their intuition nurtured through years of experience, these wholesalers have grown worried about the recent trend of harvesting tiny sea cucumbers in Japan. For example, in March 2005, Charlie Lim, the secretary general of the SMPA (Sharkfin and Marine Products Association), indicated that Japanese dried sea cucumbers were arranged according to 15 size-based types in Hong Kong. He noted that sea cucumbers that were classified 5 through to 1 (Table 22.5)

Table 22.5 Classification of Japanese Dried Sea Cucumber in Hong Kong Markets

Classification	Number of Pieces per 600 g	Weight per Piece (g)
1	20–30	20–30
2	30–40	15–20
3	40–50	12–15
4	50–60	10–12
5	60–70	8.6–10
6	70–80	7.5–8.6
7	80–100	6.0–7.5
8	100–120	5.0–6.0
9	120–150	4.0–5.0
10	150–180	3.3–4.0
11	180–220	2.7–3.3
12	220–250	2.4–2.7
13	250–300	2.0–2.4
14	300–350	1.7–2.0
15	350–400	1.5–1.7

From an interview with Charlie Lim of SMPA, March 2005

had been popular until around 2000, but that small sea cucumbers weighing about 1 g had recently become common.

Lim suspected that a possible reason for this shift in trends was that large sea cucumbers had decreased in numbers owing to overexploitation. Another possible reason, according to Lim, might have been changes in consumption trends. In the past, large sea cucumbers were in demand, but more recently, sea cucumbers ranging between classification 7 and 11 (Table 22.5) started selling well compared to large ones. In the past, the popular custom was to cook large thick sea cucumbers and serve them on large plates, but around 2000, the custom underwent an evolution in Hong Kong and, more generally, in new Chinese cuisine; most sea cucumbers were smaller and served on small plates. Assuming that the retail price per catty (600 g) is 100,000 JPY (almost equal to 1,000 USD), if one catty includes only 30 pieces of large sea cucumbers, the price of one sea cucumber is equal to 3,300 JPY (about 33 USD), and when one sea cucumber is served on one plate, the price for each plate of the dish becomes very expensive. On the other hand, if one catty includes 400 pieces of small sea cucumbers, the price of one sea cucumber is equal to about 250 JPY (2.5 USD), and so the menu becomes affordable.

Lim expressed his anxiety over the current boom in sea cucumber harvesting in Japan. As shown in Section 22.4.1, the current Fishery Act in Japan does not allow the Fisheries Agency, which is the supervisory agency attending to matters of this kind, to manage local coastal operations, except fishing operations, such as whaling, that require the permission of the Ministry of Agriculture, Forestry, and Fisheries. However, Lim also acknowledged that each coastal community in Japan has its own history of resource management. For more details on local sea cucumber fisheries management in Japan, see Akamine (2009, 2013) and Makino (2011).

22.5.2 JAPANESE SALT-PRESERVED SEA CUCUMBERS EXPORTED TO DALIAN

While visiting coastal communities in Hokkaido and Aomori prefectures in 2007, the author of this chapter came across sea cucumber buyers from Dalian, China. These buyers not only brought about changes in distribution routes but also changes in production methods. For example, merchants started shipping salt-preserved semidried products, rather than “traditional” dried products, especially in Aomori Prefecture. Processors produce the semidried products by gutting sea cucumbers, boiling them, and adding salt to them. As a result, salt-preserved sea cucumbers accounted for 80% of the 1600 tons of captured sea cucumbers in Aomori Prefecture in 2007, according to an estimate by Dr Hirota Masahito of the Fisheries Research Center.

The production of marketable dried sea cucumbers requires sophisticated and advanced techniques. However, starting up a business that deals in salt-preserved sea cucumbers is quite easy as long as sufficient capital is available. According to an analysis by Dr Hirota, many scallop (*Mizuhopecten yessoensis*) processors established salt-preserved sea cucumber businesses in Aomori Prefecture in the early 2000s. These startups emerged probably because the price of scallop had dropped and the price of fresh sea cucumber had skyrocketed in 2007. So it became reasonable for processors to produce salt-preserved sea cucumber, with minimum risks and a high turnover of funds, rather than producing dried sea cucumber, a task that carries with it a high risk of yielding defective products. Against this backdrop, people in Hokkaido have also been expressing a growing interest in salt-preserved sea cucumbers.

The yield rate of gutted, boiled, and salted sea cucumbers is 15–20%, meaning that 80–85% of the original catch (fresh weight of whole sea cucumbers) is waste material. Therefore, by purchasing salted sea cucumbers, Chinese businessmen can drastically reduce shipping costs because the weight of these sea cucumbers is relatively small. Indeed processors in Aomori Prefecture and Hokkaido have stated that, as far as they can tell, the salt-preserved sea cucumbers they have been producing are being further processed into dried sea cucumbers and other products in China. Chinese businessmen are able to cut personnel costs related to gutting and boiling, which constitute the second-greatest outlay of funds after expenses associated with production ingredients. By reprocessing the sea cucumbers in China, where manpower is available at low cost, the business is profitable even if the cost of ingredients is relatively high.

22.6 PROBLEMS AND PROSPECTS IN SUSTAINABLE *A. japonicus* PRODUCTION IN JAPAN

Dr Hirota's observation on shifts of production from dried sea cucumber to salt-preserved sea cucumber in Aomori Prefecture in 2007 may also be applied in other prefectures across the Japanese archipelago. This conjecture partly explains the drop in dried sea cucumber exports to Hong Kong from Japan after 2007.

There is a notable difference between the distribution methods for traditional dried sea cucumber and the distribution methods for newly introduced salt-preserved sea cucumber. In the case of dried products, there is the Nam Pak Hong–Yide Lu route developed over the course of many long years of business, and wholesalers in Japan and Hong Kong directly trade dried products based on trusted relationships. In this way traders in both countries can understand the overall flows of products even though neither Japanese nor Hong Kong traders disclose the details of their network to each other. This is possible because, first of all, there is a limited number of processors who can produce high-quality dried sea cucumbers, and so poachers operating outside the network are easy to trace. In contrast, the newly introduced salt-preserved sea cucumber can be produced by most seafood processors insofar as no sophisticated experience is required, and it is thus difficult to clarify their domestic distribution and how the exported product is consumed in each country. The most pressing problem is that as salt-preserved sea cucumber became popular, an increasing number of fishermen capture sea cucumber during closed seasons and capture undersized animals; two activities that are strictly illegal. This is one of the most serious problems that the Japanese fisheries community is facing today.

Both processors and fishermen try to stimulate demand for all types of sea cucumber commodities. Some have developed new recipes, such as sea cucumber dumplings, sea cucumber *chazuke* (cooked rice with sliced sea cucumber, pouring green tea on it), or sea cucumber *udon* (powdered sea cucumber kneaded in noodles). All of them are still tentative. Some famous producing areas even host sea cucumber festivals, such as Yokohama in Aomori Prefecture and Noto in Ishikawa Prefecture. Since 2010, Noto Sea Cucumber Processor's Association began to hold sea cucumber memorial services, expressing gratitude and dedication to sea cucumbers, when they close the sea cucumber season in late March (Figures 22.11 and 22.12). Memorial services for fish and marine animals are commonly practiced throughout Japan but the only one exclusively dedicated to sea cucumbers is found in Noto. Before the memorial service, the organizer holds a symposium on sea cucumbers, inviting all stakeholders to attend. The ceremony can serve as a visible symbol for gathering together different stakeholders, including local government representatives, fisheries associations, and associations of processors and managers.

FIGURE 22.11

Sea cucumber memorial service in Noto, Japan in March 2010.

Photo by J. Akamine

FIGURE 22.12

People getting ready to release young sea cucumbers in the ocean along the coast of Noto, Japan in March 2010.

Photo by J. Akamine

People in Noto release young sea cucumbers along the coast after the memorial service. Young children are invited to release sea cucumbers. This is because the Noto Sea Cucumber Processor's Association wants young people in Noto to be interested in natural resources, such as sea cucumbers.

Stocking sea cucumber is common in many parts of Japan. They release local seedlings of sea cucumbers produced in aquaculture facilities. Chinese buyers prefer spiky varieties but one should bear in mind that Japan has rich genetic resources of *A. japonicus* from spiky to less spiky, each with many color variants. Compared to neighboring *A. japonicus*-producing nations, this is what sets Japan apart, and there is a strong will to conserve such a rich genetic resource for the future.

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CHAPTER

Apostichopus japonicus: *"DOLGI HAESAM"* IN THE REPUBLIC OF KOREA (SOUTH KOREA)

23

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SUMMARY

Sea cucumbers in the Korean Peninsula have been well known as nutritious and wholesome seafood, often called "ginseng of the sea." A number of edible color variants of *Apostichopus japonicus* are harvested along Korean coastal areas and conventionally divided into red, blue, and black sea cucumbers. The annual catch of wild sea cucumber in the Republic of Korea decreased from the early 1980s due to an increase in fishing pressure, the deterioration of the coastal environment by pollution, climate change, and habitat loss driven by land reclamations. Since the mid-2000s, however, sea cucumber production began to increase mainly in response to new management measures implemented by the government, such as the release of seedlings grown in aquaculture facilities. As a health food, with an eco-friendly image, sea cucumber has a great potential for exports to foreign markets, especially in neighboring countries, such as Japan and the People's Republic of China. Thanks to the governmental initiatives and increasing demand in and outside the country, sea cucumber aquaculture is expected to grow rapidly in the future.

Keywords: *Apostichopus japonicus*; aquaculture; culture technique; dolgi haesam; haesam; fish farming; fisheries; research and development; sea cucumber; Republic of Korea

23.1 TRADITION

As in China, sea cucumber in the Korean Peninsula has long been considered nutritious and wholesome seafood, often called "ginseng of the sea" since the late Choson Dynasty (1392–1897). It is speculated that such a perception of sea cucumber would originate from its beneficial biochemical characteristics – unique and strong alkalinity – rather than its richness in nutrients (with the exception of its mineral contents) (Kim, 1993) (see also Chapter 19).

Despite the lack of decisive archeological evidence, the origin of sea cucumber consumption in the Korean Peninsula is believed to go back to at least the Neolithic age. In the Dongsam-dong shell mound in Busan, one of the most well-known prehistoric sites along the southern coastal area of the Peninsula, which began to be excavated in the early 1970s, remains of sea urchin (an echinoderm relative of sea cucumber), as well as shellfish and fish species were discovered (Kang et al., 2000). Similarly, the

Park, S., Hong, S., Yoon, I., Lovatelli, A. 2015. Chapter 23. *Apostichopus japonicus*: "Dolgi Haesam" in the Republic of Korea (South Korea). In: Yang, H., Hamel, J.-F., Mercier, A. (Eds.), The Sea Cucumber *Apostichopus japonicus*. History, Biology and Aquaculture, Academic Press, pp. 423–432. http://dx.doi.org/10.1016/B978-0-12-799953-1.00023-4

Dongsam-dong site shows indirectly that residents in that period (~5000–4000 B.C.) should have enjoyed sea cucumber as well. This is because sea cucumber and sea urchin are considered to share a common habitat with roughly the same preferred environmental conditions and would have been collected and consumed by the same people. However, no traces were found, probably because, contrary to sea urchins, sea cucumbers lack large hard skeleton structures and are not well represented in fossil records.

Ancient Korean literature provide evidence that the tradition of enjoying sea cucumber as a rare seafood item among other favorite seafood species has a long culinary history in the region. In particular, throughout the history of the Choson Dynasty, descriptions related to harvesting and consumption of sea cucumber are frequently found in numerous publications, such as the geographic encyclopedias printed by the Dynasty's official compilations, as well as collections of academic and/or literary works written, in Chinese character, by individual scholars called *sadaebu* (literati).

For example, *Chŏn'ŏji* (Encyclopedia of Fisheries Management)[1] written by a famous natural historian called Yugu Suh[2] in the early nineteenth century depicts not only the morphological traits of sea cucumber, but also its popularity and traditional processing method. According to this description, sea cucumbers were lightly scorched and sun-dried before they were sold by specialized vendors in traditional market places. According to this book, 10 pieces of dried sea cucumber were placed on a skewer for customers to choose and purchase. The book refers to sea cucumber as "the most outstanding seafood beneficial to human health" (Kim, 2007).

Palyŏkchangsi (Market Places of Eight Provinces)[3] provides detailed information about traditional market distribution in the early nineteenth century during the Choson Dynasty (Kang et al., 2000). Yugu Suh includes lists of sea food shipped to and traded in the local markets. From his lists, we understand that sea cucumber was a widely traded seafood item across the country, especially in the Yeongnam Province.[4]

Despite this, sea cucumber was never ranked as a major species for commercial production in the Korean Peninsula, not even during the Japanese colonial rule (1910s–1930s) when the fisheries sector traditions and data recording were revisited, nor following the liberation in 1945 and the Korean War (1950–1953). During the Japanese ruling of the fisheries industry, it focused mainly on fish species, such as sardines, the Alaskan pollack, mackerels, and anchovies. Recently, consumption of sea cucumber has increased particularly as an appetizer (typically with drinks ordered from outdoor pushcart stalls) or as a complimentary side dish when ordering halibut or the Korean rockfish (*Sebastes schlegeli*) at exclusive sushi restaurants.

23.2 POPULATION DISTRIBUTION, PRODUCTION, AND MANAGEMENT

Even though there are about 1200 species of sea cucumber across the globe, only one is known to be harvested along the Korean coastal belt (although this species is locally divided into 14 variants; shown later), especially in South Korea (National Fisheries Research and Development Institute (NFRDI),

[1]More precisely, *Chŏn'ŏji* is the title of one chapter from *Imwŏnsibyukji*, an extensive encyclopedia on how to provide for a healthy rural life, covering 16 different essential rustic areas including agriculture, horticulture, livestock farming, house maintenance and repairs, education, medicine, etc.

[2]In terms of Korean history, Suh is best known for *Shilhakpa* (the School of Pragmatism).

[3]*Palyŏkchangsi* is another chapter of *Imwŏnsibyukji*. The eight provinces encompass virtually all of the country as the Choson Kingdom consisted of eight provinces.

[4]It includes current South and North Gyeongsang provinces.

Incheon Regional Maritime Affairs and Fisheries Office, 2006). These variants are divided into three main groups and known as: *Hong haesam*, the red sea cucumber, *Ch'ŏng haesam*, the blue sea cucumber, and *Hŭk haesam*, the black sea cucumber[5] (see Chapter 3 for *Apostichopus japonicus* color variations in China; although the blue variant is not described in China, it might have to do with local perception and could be equivalent to the green variety described there). This conventional classification is based on different elements, such as body color, distribution, and habitat. All are believed to be variants of the Japanese sea cucumber (*A. japonicus*) but are locally referred to as distinct "species."

Almost all the variants have been, until recently, harvested from the wild. According to a study conducted by the Korea Maritime Institute (KMI) in 2012, production of sea cucumber from fisheries accounted for 96.1% of the total production, while only 3.9% derived from mariculture practices in 2011 (Lee, 2012).

The red variant of *A. japonicus*, which can cope with strong tides and severe environmental conditions, is cultivated in aquaculture facilities exclusively on Jeju Island (the largest and southernmost island in the Korean Peninsula). Other color variants are distributed in bays and/or shallow mud flats along the coast of the Yellow Sea, locally known as the West Sea (*Sŏhae*), the East Sea (*Donghae*), and the South Sea (*Namhae*).

Until recently, sea cucumber harvesting was mainly dependent on traditional methods, despite the introduction of improved diving equipment during the Japanese rule, which consisted of skilled female divers (*haenyŏ*)[5] or community-based fisheries (*Maŭl Ŏ'ŏp*)[6]. For instance, on Jeju Island, sea cucumber harvesting has been carried for several decades by middle-aged or aged female divers. Generally, the production of sea cucumber from these fishing communities is undertaken through a cooperative's management supervision and supported by the government in the form of seedling restocking programs (Lee, 2012). The sea cucumber harvesting season begins in December and ends in May or June of the following year (Kang et al., 2000).

In 1983, the catch of sea cucumber reached its highest production level at 3966 tons (Kang et al., 2000). Since then, annual catches of wild sea cucumber have gradually decreased mainly due to an increase in fishing pressure (typically undertaken by small-scale fishers), to an overall deterioration of the coastal environment, habitat loss driven by land reclamations, and possibly also due to climate change impacts. For instance, the harvest of about 2500 tons in 1990 dropped to 1614 tons in 2006 (Figure 23.1).

Since the mid-2000s, however, sea cucumber production in the Republic of Korea began to experience a major turning point, which has been mainly initiated by a decision of the government to designate sea cucumber as a priority species requiring a dedicated resource management program.[7] In order to ensure further development of this benthic fishery, the government has engaged itself in supporting

[5]*Haenyŏ* is a unique tradition only in Korea and Japan dating back over 2000 years and historically to the Koryo Dynasty according to the official annals written in 1105. *Haenyŏ* appears to have originated from Jeju Island where most of the 20,000 Korean *haenyŏ* resided. A *haenyŏ* typically starts swimming before the age of 10, training in her teens, reaching the peak of her career around 40, and retiring at about 60. *Haenyŏ* typically possess special gathering skills and mental strength, being able to remain for about 2 min at 20 m depth even in cold winter waters (encykorea.aks.ac.kr; doopedia.co.kr, both accessed on January 22, 2014).

[6]*Maŭl* means "community," and *Ŏ'ŏp* means "fisheries." According to the Fisheries Act in the Republic of Korea, *Maŭl Ŏ'ŏp* (community-based fisheries) is defined as the "business run by a fisher residing in a certain locality to manage, cultivate, catch and gather shellfish, seaweed or sedentary marine animals in a demarcated area of waters, contiguous to the shore, within a certain depth range."

[7]Major harvesting areas in South Chungcheong Province are Taean County and Boryeong City. For more details, see Lee (2012).

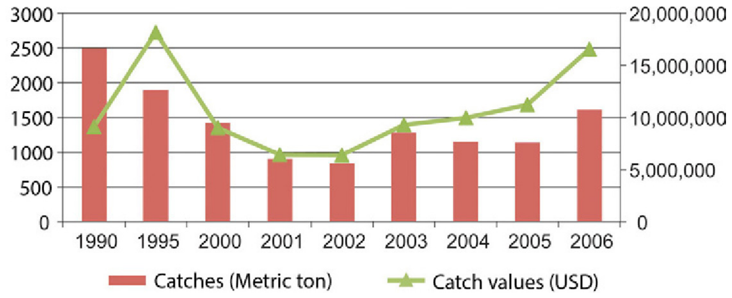

FIGURE 23.1

Annual catches of *A. japonicus* in the Republic of Korea. Note: Catch values were originally collected in Korean won and converted to US dollar based on the average exchange rates at closing prices in the respective years.

From Ministry of Food, Agriculture, Forestry and Fisheries

a relatively large restocking program, releasing artificially reproduced seedling in targeted areas. This program started in 2003 (National Fisheries Research and Development Institute, 2010). As shown in Figure 23.1, following this new management practice, the annual growth of sea cucumber catch stopped decreasing and started increasing again.

In recent years, the annual sea cucumber production has been fluctuating between 2300 and 3000 tons (wet weight). As mentioned earlier, sea cucumber is harvested along the entire coastal area of the Republic of Korea. In 2011, South Gyeonsang Province (including Tongyeong City) accounted for the largest output (75%) of the total production with 1005 tons (Park et al., 2013). South Chungcheong and South Jeolla provinces followed with 686 and 161 tons, respectively. An outstanding production level has been attained in the South Chungcheong Province with a production increase from just under 40 tons in 2006 to over 10 30 tons in 2010 (in 2011 the production dropped to 686 tons).[7]

23.3 CONSUMPTION AND COOKING RECIPES

Many scholars during the late Choson Dynasty described the biological and morphological traits of sea cucumber, but also introduced various recipes on how to prepare it for consumption. Yakjeon Jeong (1758–1816), a famous scholar exiled to *Jasan* Island (also called *Hŭksan*) in the Shin'an County, South Jeolla Province at the beginning of the nineteenth century, wrote his fish dictionary, *Jasan Ŏbo*. He mentioned that dried sea cucumber is an appetizing marine product including it as one of the "three highly regarded seafood items along with abalone and mussel" (Jeong, 1998). In the *Chŏn'ŏji* encyclopedia, Yugu Suh comments on salted sea cucumber intestines (known as *konowata* in Japanese) as "an excellent side dish when drinking," introduced in the Korean Peninsula eating habits by the Japanese (Kim, 2007). Considered as a gourmet food, *konowata* is not only consumed on its own but is also eaten on sashimi or rice.

Traditional recipes for sea cucumber were passed down to present-day Koreans who currently enjoy and consume the echinoderm served in Chinese Korean-style restaurants (preparations usually from dried sea cucumber) or in Japanese sashimi restaurants (as sliced fresh sea cucumber). The sea cucumber is also consumed as seasoned dishes with vinegar and other condiments

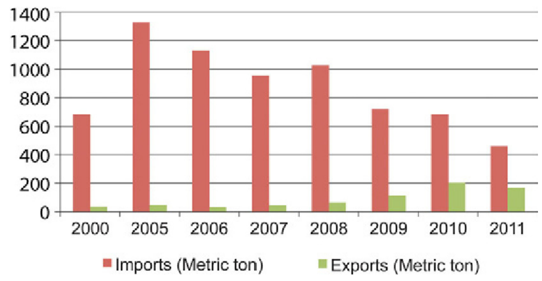

FIGURE 23.2

Annual imports and exports of sea cucumber in the Republic of Korea. Note: The total volume of sea cucumber trade includes salted, frozen, dried, refrigerated goods, and other products.

Adapted from Lee, 2012

(*hoemuch'im*) or as *konowata* when seasonally available. Sometimes sea cucumber is enjoyed as a steamed product mixed with ground meat, pheasant meat, chicken, abalone, and shiitake mushrooms (Han, 1998).

23.4 IMPORTS AND EXPORTS

Mainly due to a relatively small production, the price for sea cucumber has remained high. In recent years, the sea cucumber price rose from 8.5 USD kg^{-1} in 2008 to 13.9 USD kg^{-1} fresh weight in 2011. This rapid increase is believed to have been triggered and affected by a combination of increased Chinese domestic demand of the locally produced and harvested sea cucumbers (Lee, 2012).

Until the mid-2000s, imports of sea cucumber exceeded exports with more than 1000 tons annually. The balance of import and export changed in the late 2000s with total imports decreasing from 1027 tons in 2008 to 702 tons in 2009 with a further drop to 461 tons in 2011. Over the same period, the exports increased by more than 100 tons, a significant increase compared to less than 50 tons in the early 2000s (Figure 23.2). Increases in exports are even more obvious in terms of trade values. The export values of sea cucumber in 2011 reached 13,086,000 USD compared to 302,000 USD in 2000 – equivalent to a 43-fold increase. In contrast, the import values decreased by threefold during the same period. Ninety-three percent of the exports in 2011 went to China, including the Special Administrative Region of Hong Kong (Lee, 2012).

23.5 RECENT DEVELOPMENT OF SEA CUCUMBER FARMING AND SOME PROSPECTS

In the early 2000s, the government of the Republic of Korea listed the sea cucumber *A. japonicus* as one of the "top national favorite seafood items" and designated it as one of the 10 exportable strategic species for research and development. Various factors have led to an increased attention to sea cucumber. First, it is perceived and prized as a health food by Koreans. Second, its eco-friendly image has been promoted by national campaigns for environmental protection; the sea cucumber inhabits clean

Table 23.1 Sea Cucumber Seedling Production in the Republic of Korea by Local Governmental Research Institutes in 2010

City and Province	Surface Area (m^2)	Annual Production (Number of Juveniles)
Incheon	50	10,000
Gangwon Province	20,817	300,000
Chungnam Province (South Chungcheong)	300	10,000
Jeonbuk Province (North Jeolla)	50	70,000
Jeonnam Province (South Jeolla)	720	1,000,000
Gyeongnam Province (South Gyeongsang)	140	35,000
Gyeongbuk Province (North Gyeongsang)	172	240,000
Jeju Province	1,204	80,000
Total	**23,453**	**1,745,000**

Adapted from Inchon Metropolitan City, 2011

water and feeds, at least in part, on macrophytes (see Chapter 10). However, more than anything else, sea cucumber is believed to have a great potential for export to foreign consumers, especially China, and therefore constitutes a promising source of income for the local fishing communities.

The rapid growth of the sea cucumber aquaculture subsector in China earlier this century and the significant increase in demand for sea cucumbers by Chinese consumers have certainly encouraged the development of the same aquaculture industry in the Republic of Korea. By 2005, the number of sea cucumber seedlings produced artificially in China had reached about 22 billion (see Chapters 2 and 16) with aquaculture production exceeding 150,000 tons (see Chapter 20). All these factors led to investment of time and effort, and money for more production, and triggered a boom in the research and development of sea cucumber farming in the country.

Since 2003, over 200,000 sea cucumber seedlings haven been released annually for stock enhancement programs in the Republic of Korea.[8] It was, however, soon realized that this production was far too small to satisfy even a single sea cucumber farming facility, which led the authorities to appreciate the importance of increasing the output of artificially produced seedlings if the industry was to grow (Table 23.1). In order to achieve this goal, governmental research institutes started a series of applied research projects. For example, the West Sea Fisheries Research Institute (a branch of the NFRDI located in the Yellow Sea region) conducted projects on artificial seed production and integrated multitrophic farming methods from 2003 to 2006. The work carried out by the Institute included tests on growth improvement and early-stage sea cucumber seedlings' survival, growth trials on 1-cm-long juveniles less than a year old, as well as polyculture trials with abalone on land-based facilities. Furthermore, guidelines for stock enhancement activities were developed and promoted.[9]

[8]In South Korea, the first successful technical development for artificial seed production of sea cucumber took place in 1990 in the Pohang branch of the National Fisheries Research and Development Institute (NFRDI).
[9]The final outcome of the research project was reported by National Fisheries Research and Development Institute (2010).

Table 23.2 Summary of Farming Technologies for the Sea Cucumber *A. japonicus* in the Republic of Korea, China and Japan

Country	Production Technology of Artificial Seed	Developmental Stages in Terms of Aquaculture System		
		Land-Based Tank	Coastal Pond	Seed Releasing
Republic of Korea	Settled production technology (30 million seeds in 2006)	Initial R&D stage for industrialization (2006)	Initial R&D stage for Industrialization (2006)	Mid-R&D stage for industrialization (2006)
China	Mass production technology (2.18 billion seeds in 2005)	Settled R&D stage for industrialization	Settled R&D stage	Settled R&D stage for industrialization
Japan	Settled production technology (3.36 million seeds in 2004)	Mid-R&D stage for industrialization	—	Settled stock recovery stage

In this process, and as part of a larger national development program, a comparison on sea cucumber farming advances between a number of countries in the region (mainly China – see Chapters 16 and 17 – and Japan Chapter 22) revealed that in the Republic of Korea there was an important technological gap in the farming technology compared to its neighboring countries, particularly in the early stages of sea cucumber larval development (Table 23.2).

Although the national production of sea cucumber remained rather low with 92 tons produced in 2011, accounting for 3.9% of the total national production (Table 23.3) and the aquaculture industry still in an infancy stage, the sea cucumber aquaculture sector is expected to rapidly grow for several reasons as outlined further on. First, the central government is playing an active role in supporting and promoting the growth of fisheries' export markets. In 2012, the government decided to support the sea cucumber industry, listing it as one of the "top 10 strategic species in the fisheries sector for industrialization," which includes species such as tuna, flounder, abalone, oyster, laver, and sea mustard (algae). Second, local governments in the country have also expressed a keen interest in expanding sea cucumber farming even though further decentralized research and development support is needed. For example, Incheon City announced in 2011 that it would support the establishment of a sea cucumber processing facility, through technical exchanges with Chinese experts, and attract Chinese investments (Shin, 2011). It is likely that in a foreseeable future such local efforts will narrow technological gaps between the country and its neighbors, expanding Korean sea cucumber production capabilities (Table 23.2). Third, the international demand for sea cucumber has increased. Over the past decade, although major sea cucumber-producing provinces in China, such as Liaoning and Shandong, have increased their production (see Chapters 16 and 21) by also converting shrimp ponds into sea cucumber farms, the current supply does not meet the demand in the country (Park, 2011).[10] In 2010 the total market volume of sea cucumber in China was estimated by the Korean authorities to be at 4.96 billion USD. Consequently, South Korea's export potential of sea cucumber, especially aimed at China, will

[10]For three years in the late 2000s, areas of sea cucumber farming in China increased about three times from 58,000 ha in 2006 to 155,000 ha in 2009.

Table 23.3 Annual Catches of *A. japonicus* by Various Fishing Techniques in the Republic of Korea (Lee, 2012)

Categories		2010		2011			
		Catches (Metric Ton)	Values (USD)	Catches (Metric Ton)	Catches Proportion (%)	Values (USD)	Values Proportion (%)
Total		**2,687**	**28,802,768**	**2,351**	**100.0**	**31,798,736**	**100.0**
Fishery	Subtotal	2,687	28,802,768	2,259	96.1	30,082,130	94.6
	Diving fishing with equipment	544	6,153,114	631	26.8	7,524,368	23.7
	Coastal fishing with traps and pots[a]	73	720,588	103	4.4	1,209,386	3.8
	Communal fishing	460	5,256,055	748	31.8	12,510,830	39.3
	Others	1,610	16,629,758	777	33.0	8,836,643	27.8
Aquaculture		—	—	92	3.9	1,716,606	5.4

Note: Values in 2010 and 2011 were originally collected in Korean won and converted to USD based on the average exchange rate at closing prices in the respective years (1156 KRW to 1 USD in 2010, 1108 KRW to 1 USD in 2011).
While traps are large structures fixed to the shoreline, pots are smaller, movable traps, enclosed baskets or boxes that are set from a boat or by hand (see FAO, 2001).

obviously depend on increasing its production capability, which will be driven by the development and adoption of sea cucumber farming technologies and knowledge exchange with neighboring countries, such as China.

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CHAPTER

Apostichopus japonicus IN THE DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA

24

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SUMMARY

This chapter provides a brief introduction to the history of consumption and fishery of the Japanese sea cucumber, *Apostichopus japonicus*, in the Democratic People's Republic of Korea (i.e., North Korea). Commercial fishing of this holothurian species dates back to the early fifteenth century; collection was mainly by hand by women skin divers. Fishing intensified in the early 1900s with the introduction of SCUBA equipment, which led to a gradual decline of the wild resources. This chapter also presents information on the recent development of aquaculture programs aimed at restoring the natural resources and boosting production, both for the domestic markets and the lucrative export markets.

Keywords: *Apostichopus japonicus*; aquaculture; fishery; markets; Democratic People's Republic of Korea (North Korea); sea cucumber

24.1 INTRODUCTION

Harvesting and culture of marine species have traditionally played important roles in the livelihood of many coastal communities of the Democratic People's Republic of Korea (i.e., North Korea) where these activities contribute significantly to the economy and food security (FAO, 2006). The Koreans have been collecting sea cucumbers for human consumption for many centuries. Historical records (annals) dating back to King Sejong (1418–1450; Joseon Dynasty) and the historical book *Dongguk Yeoji Seungram*[1] record in great detail the catching and consumption of sea cucumbers in the different administrative regions of Joseon Korea. The records indicate that the capture of this benthic organism for commercial purposes dates back to the early fifteenth century throughout the Korean Peninsula and that women divers were the first to harvest them. Since then, sea cucumber has become an important commercial marine seafood

[1]A book from the Sinjeung Dongguk Yeoji Seungram (新增東國輿地勝覽), a series of geography books published in Joseon Korea in 1530. The books described the different administrative regions of Joseon Korea, including their geography, history, customs, government offices, temples, shrines, fortresses, local products, and places of scenic interest.

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species for both the export and internal markets, with production volumes steadily increasing over the years. Dried sea cucumber (also known as "beche-de-mer" or "trepang") constitutes the main processed form.

People in Democratic People's Republic of Korea have regarded sea cucumber to be as valuable as the ginseng root (locally known as *insam*) with comparable therapeutic properties. It is consumed and served as a delicacy to important guests as a demonstration of appreciation and courtesy. When used for medical treatment, the consumption of this holothurian is traditionally believed to help "purify the blood" from toxic body substances.

24.2 FISHERY

Japanese sea cucumbers (*Apostichopus japonicus*) were traditionally caught by hand by women divers until the fifteenth century, and by spear-fishing around the seventeenth century. Starting in 1860, with the presence of the Japanese on the Korean Peninsula, diving apparatuses to collect sea cucumbers were introduced, and by the start of the 1900s records indicated that 120 diving apparatuses were available and in use. At the beginning of the 1990s the decline in the fisheries' resources and shortage of fishing gear contributed to the overall decline in domestic fish production, well below the minimum subsistence level (FAO, 2006).

The Japanese sea cucumber has been fished throughout the coastal waters of Democratic People's Republic of Korea with abundant stocks found intermittently, depending on sea topography, along most of the east coast (Sea of Japan) and West Sea (Yellow Sea). On the east coast areas around Kosong, Tongchon, Kajin, Jongpyongsamho, Soho, Rakwon, Hongwon, Sinpo, Sinchang, Riwon, Kimchaek, and others, sea cucumbers have been fished in abundance. They are also found in large numbers around the Ongjin and Cholsan peninsulas and Jangsan Point, off the western coast of the country. Unfortunately, and despite the absence of statistical data and information on the status of the wild stocks and the quantity fished, it has been recognized that landings of this valuable holothurian have decreased significantly since the early 1990s, most probably due to heavy exploitation and inadequate management of the resource.

There are currently no conservation zones (i.e., no-take zones or marine protected areas) in Democratic People's Republic of Korea to protect sea cucumbers from overexploitation. Authorized fishing enterprises nevertheless apply some conservation measures in their concession areas to attempt exploitation of the wild sea cucumber resources sustainably. In general, fishing enterprises operate and manage grounds of several hundred hectares where no particular regulations are in force with regard to the exploitation of the sea cucumber. In general terms, the enterprises attempt not to overfish their stocks to allow sufficient natural recruitment and maintenance of the holothurian population at levels that allow regular landings.

In general, sea cucumber fishing is loosely prohibited from September to March while permitted from May to August. Bottom trawling for sea cucumber is however strictly prohibited, particularly over grounds that have the right habitat for the holothurian; there is no restriction on the use of other fishing methods or gear. Landings of sea cucumbers measuring less than 8 cm in length are prohibited.

24.3 AQUACULTURE

In the recent past, sea cucumber aquaculture was poorly developed in the country. The Raksan Experimental Station belonging to the East Sea Fishing Research Institute was the only facility conducting small-scale research trials on sea cucumber culture from 1975 to 1980, in an effort to

FIGURE 24.1

Sea cucumber hatchery in the small Hongwon Fishing and Aquaculture Cooperative.

Photo by Alessandro Lovatelli, FAO

study the biological characteristics and reproduction biology of *A. japonicus*. In the 1990s interest in sea cucumber aquaculture increased significantly as the demand for beche-de-mer and other derived products had increased both internally and in neighboring countries, as well as to help restore wild stocks that had been intensively exploited.

Commercial sea cucumber aquaculture operations in Democratic People's Republic of Korea only commenced in 2000, during which time the government had set high production goals and hence supported the introduction of improved production systems and technologies, and facilitated technical capacity building through the assistance of the Food and Agriculture Organization of the United Nations (FAO, 2006). At present, sea cucumbers are being intensively cultured in some localities, although the overall national production level remains low and accounts for less than 1% of the total annual aquaculture output. Bivalves and seaweeds account for most of the aquaculture production in the country.

As sea cucumber aquaculture in Democratic People's Republic of Korea is still in its infancy, only a few dedicated commercial facilities are in operation, mainly due to the limited amount of juveniles being produced (Figure 24.1). Local breeding technology has nevertheless improved with the introduction of reproduction protocols from China where the farming of the same species is well developed and successful (Figure 24.2). There is no great difference between the sea cucumber culture methods in Democratic People's Republic of Korea and China, except that Chinese companies have succeeded in producing and supplying quality feed, particularly during the early growth stages that enables Chinese companies to produce large volumes of seed material, which are mainly released at sea for on-growing (see Chapters 7, 8, and 15 for details on the Chinese culture of *A. japonicus*).

Areas around which *A. japonicus* culture facilities are concentrated in Democratic People's Republic of Korea include Sinchang Bay and Rajin Bay in Musudan and Hwadae counties off the East Sea coast, around Ryongyon Bay in Kangryong Peninsula, Kirin and Changrin islands in Ongjing County, and in Taegyedo in Kwaksan County. The reason why these facilities are concentrated in these areas is simply because they provide logistical advantages (e.g., availability of labor) and accessibility (roads) and availability of fish landing facilities. Nonetheless, almost all the coast to the east and west provide adequate culture sites for *A. japonicus*.

FIGURE 24.2

Rearing of juvenile sea cucumbers, *A. japonicus*, shown here feeding on benthic diatoms.

Photo by Alessandro Lovatelli, FAO

Sea cucumber culture on a large industrial scale in Democratic People's Republic of Korea is still in its infancy. In some localities large artificial ponds of several hectares have been created for the growing on of *A. japonicus*, such as those established by the Ryongyon Fish Farm in Ryongyon County, North Hwanghae Province. In other localities, sections of relatively protected sea areas have been diked to form large water reservoirs (e.g., the Kwaksan Seawater Reservoir) where sea cucumbers are released and left to grow. At the Kimchaek May 24 Taegyong Fish Farm, discarded scallop shells are being tested in culture ponds as a substrate for *A. japonicus* to grow on (see Chapter 18 for details on artificial reefs). Juvenile sea cucumbers are raised in protected areas until they reach a length of about 2–3 cm before they are released at sea or in the larger grow-out ponds.

Starting in 2012, the North Korean farms adopted the Chinese technique of preparing the grow-out areas prior to the release of the hatchery-produced juveniles. This basically consists of laying rocks at the bottom of the growing on ponds and selected sea sites. Rocks are piled up to form complex structures that allow the sea cucumbers to find adequate refuge and larger feeding areas; they are generally spaced 50–100 m apart from each other, often covering an area of dozens of hectares. In some localities where sea cucumbers have been released, seaweed long-line aquaculture is also carried out over the farmed area to further stimulate the growth of the holothurians feeding on detritus falling from above (Figure 24.3).

Processed *A. japonicus* is sold in the markets of all major cities in Democratic People's Republic of Korea, including in the well-known Pothonggang Seafood Shop in Pyongyang. The sea cucumbers are sold mainly as dried products (beche-de-mer), but also frozen or preserved in salt. Other typical products include processed viscera and an alcoholic drink simply known as "sea cucumber liquor."

Over the past decade, the Food and Agriculture Organization of the United Nations has actively assisted in the transfer of farming technologies of several commercially important marine organisms, including the Japanese sea cucumber. Technology acquisition is still ongoing through a series of national capacity-building activities. The expansion and industrialization of sea cucumber farming in

FIGURE 24.3

View of the area allocated to the Hongwon Fishing and Aquaculture Cooperative. Sea cucumber juveniles are released for growing on under the kelp seaweed long-lines.

Photo by Alessandro Lovatelli, FAO

the Democratic People's Republic of Korea is a matter of time and is likely to occur when the overall economic status of the fishing cooperatives has improved and the culture technology is fully acquired and applied.

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